JOURNAL

OF

HELMINTHOLOGY

Edited by
R. T. LEIPER, M.D., D.Sc., F.R.S.,
Professor of Helminthology in the University of London

Vol. VIII., 1930

Published from the
London School of Hygiene and Tropical Medicine,
Keppel Street (Gower Street),
London,
W.C.1.

CONTENTS OF VOLUME VIII.

No. 1 (February, 1930).

No. 2 (June, 1930).

Contents.

No. 3 (September, 1930).

No. 4 (December, 1930).

Journal of Helminthology, Vol. VIII., *No.* 1, *February*, 1930, *pp.* 1-18.

An Account of a Survey of the parasitic helminths of some Domestic Animals in Mid-West Wales.

By E. ANEURIN LEWIS, M.Sc., Ph.D.

(*Demonstrator in Zoology, University College of Wales, Aberystwyth, and Field-Officer to the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

INTRODUCTION.

THIS survey is a continuation of that carried out by Morgan (1924-25). The area covered, however, is more extensive, and the investigation extends over a period of four years (October, 1924 to October, 1928).

The area includes, roughly, the Parliamentary County of Cardigan, which has an exposed wind-swept sea-board, with a hinterland which rises gradually to a *High Plateau* (1,300 to 1,800 ft. above sea-level). Adjacent to the coast of Cardigan Bay is that belt of high land of a uniformly lower level (ranging from 500 to 900 ft. above sea-level) than the *High Plateau;* and known as the *Coastal Plateau.* These highlands are intersected by numerous sheltered valleys, particularly those of the rivers Rheidol, Ystwyth and Aeron.

Situated in the North-west corner of the County is a stretch of low (about sea-level) marshy ground known as *Borth Bog*, which occupies an area of about 9,150 acres. Another bog, the *Tregaron Bog*, covers some 18,000 acres. It is 500 ft. above sea-level, and situated near the Mid-west boundary of the County.

It was thought that if the whole area was divided into districts some further detailed information regarding the distribution of helminthic parasites would be obtained. It was, therefore, decided to consider that portion of land north of Aberystwyth as far as the northern boundary of the County, and stretching inland as far as the eastern boundary, as one district ; and as this included Borth Bog, it should be termed the *Borth district.* The valleys of the rivers Rheidol and Ystwyth form another,

the *Valley district;* and that stretch of land extending south of Aberystwyth as far as the southern boundary of the County, and extending from the coast to the eastern boundary as the third, or *Aeron district.*

Situated in a more or less central position of the Aeron district is the elevated ridge of *Mynydd Bach,* ranging from 1,000 to 1,183 ft. high. To the east of this ridge is *Tregaron Bog.*

This survey is based largely on the observations made, and the helminths collected from sheep, cattle and pigs at the Municipal and Llangawsa slaughter-houses, Aberystwyth. Information was also obtained from private rural slaughter-houses; from piggeries and kennels (near Aberystwyth), at which places carcases unfit for human consumption were utilised for feeding pigs and hounds; and also by visits to farms and poultry farms. Carcases and internal organs of domestic birds also were received from farmers, poultry-breeders and poultry-dealers.

An arrangement was arrived at whereby it was possible to obtain reliable information regarding the breeding and rearing places of the animals examined. The general condition of the animals was observed and the names of the farms from which the animals came were noted in a diary. No names of farms are given in the accompanying tables. These are used for reference only.

Animals brought to the slaughter-houses are usually of good (fat) butcher condition. Some, however, are of indifferent quality, while a few are bad.

Thus the evidence gleaned from the examination of these animals for parasitic helminths provides information concerning the prevalence of helminths, and acts as a guide to further enquiries regarding the economic importance of helminthic infection among domesticated animals in the area. Examination of the carcases taken to the piggeries and kennels, and of carcases and entrails sent to the writer made it possible to add to the knowledge of helminths prevalent in Mid-west Wales. Farms were visited because a certain amount of home-killing of stock is still carried out in Wales; and in order to learn more of the effects of helminths on the domestic animals.

The result of the search for helminths in sheep is given in the appended tables where the term *Spring* signifies those animals examined in April, May and June. *Other* signifies the other months of the year.

For the purposes of this survey the term lamb is restricted to those animals born about February to April, and slaughtered between April and October of the same year. All others are termed sheep.

In addition to the species of helminths from sheep recorded by Morgan, *Ascaris ovis*, *Muellerius capillaris* and *Protostrongylus rufescens* are included in the appended tables.

Observations.

Sheep.

The number of sheep examined during this investigation was 668, of which 636 or 95·2 per cent. harboured parasitic worms, and 32 or 4·8 per cent. were free from any type of helminthic parasites. The writer is of the opinion, however, that no definite conclusions can be arrived at until more animals are examined for a further number of years.

TABLE A.

Showing the Number of Sheep from the *Aeron* District Infected with the Parasites Collected.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Total.	Percentage.
Sheep examined ...	26	34	29	41	35	31	29	44	23	30	29	17	368	100
Moniezia spp. ...	7	5	8	3	3	1	17	29	4	9	9	6	101	27·4
Moniezia (young) ...	17	11	4	7	2	—	17	22	9	13	12	9	123	33·4
Monodontus trigonocephalus ...	20	31	27	40	31	24	7	9	7	5	8	11	220	60·0
Nematodirus filicollis	13	22	8	3	3	3	13	26	16	19	13	5	144	39·0
Trichostrongylus vitrinus	19	17	8	4	3	1	5	15	6	9	6	6	99	24·1
Cooperia oncophora	3	3	2	—	—	1	8	27	7	13	5	2	71	19·2
Cooperia curticei ...	6	3	1	4	3	1	2	—	—	1	1	—	22	6·0
Capillaria longipes	—	—	1	2	—	1	—	—	1	1	—	1	7	—
Ascaris ovis ...	—	—	—	—	—	—	—	1	—	—	—	—	1	—
Oesophagostomum venulosum ...	19	31	21	14	12	4	6	3	6	11	23	15	165	44·8
Chabertia ovina ...	6	3	—	1	1	—	—	—	3	3	4	4	25	6·7
Trichuris ovis ...	20	28	20	21	11	14	8	25	19	27	27	15	235	63·8
Ostertagia circumcincta	17	10	8	4	3	—	13	30	12	19	7	6	129	35·0
Hæmonchus contortus	11	6	2	—	—	—	4	6	3	5	8	1	46	12·2
Ostertagia trifurcata	1	—	1	1	1	—	—	1	1	1	—	—	7	—
Dictyocaulus filaria	6	2	1	2	1	—	1	1	4	2	6	3	29	7·8
Muellerius capillaris	5	7	—	3	3	2	1	7	3	—	8	6	45	12·2
Protostrongylus rufescens ...	2	—	—	1	5	—	1	—	—	—	—	—	9	—
Cysticercus tenuicollis	1	7	4	8	—	2	2	3	1	2	2	—	32	8·7
Echinococcus ...	—	1	—	2	11	1	—	—	—	—	2	1	18	4·9

TABLE B.

Showing the Number of Sheep from the *Valleys* District Infected with the Parasites Collected.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Total	Percentage.
Sheep examined ...	11	14	22	12	23	14	14	19	12	14	14	1	171	100
Moniezia spp. ...	7	3	3	1	—	—	2	11	8	4	3	1	43	25·1
Moniezia (young) ...	6	4	1	2	—	—	2	11	6	6	9	1	48	28·0
Monodontus trigonocephalus ...	6	13	19	10	21	7	10	8	3	5	7	1	110	64·3
Nematodirus filicollis	7	3	8	1	—	3	3	6	10	9	10	—	60	35·1
Trichostrongylus vitrinus	10	6	5	1	2	—	2	3	2	6	8	—	45	26·3
Cooperia oncophora	1	—	—	—	—	4	1	8	7	1	5	1	28	16·6
Cooperia curticei ...	7	1	1	—	—	—	—	—	—	1	4	—	14	8·3
Capillaria longipes...	2	1	—	1	1	—	1	1	2	1	1	—	11	6·4
Ascaris ovis ...	—	—	—	—	—	—	—	1	—	—	—	—	1	—
Œsophagostomum venulosum ...	10	10	19	1	6	3	2	6	2	3	8	—	70	40·9
Chabertia ovina ...	3	1	3	—	—	—	2	1	1	1	—	—	12	7·0
Trichuris ovis ...	10	9	18	6	12	4	4	12	9	11	12	—	107	62·5
Ostertagia circumcincta	7	5	9	1	—	2	3	12	11	8	10	1	68	39·7
Hæmonchus contortus	6	2	—	—	—	—	2	—	2	3	5	—	20	11·7
Ostertagia trifurcata	—	—	—	—	—	—	—	—	—	—	2	—	2	—
Dictyocaulus filaria	1	—	2	2	—	—	1	—	—	7	2	—	15	8·8
Muellerius capillaris	1	3	4	2	—	—	5	7	—	6	—	—	29	16·9
Protostrongylus rufescens... ...	—	1	1	—	6	—	—	2	—	2	—	—	12	7·0
Cysticercus tenuicollis	—	3	6	3	1	1	2	6	3	4	—	—	29	16·9
Echinococcus ...	1	2	1	—	2	3	—	1	2	—	2	—	14	8·3

TABLE C.

Showing the Number of Sheep from the *Borth* District Infected with the Parasites Collected.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Total.	Percentage.
Sheep examined ...	7	2	11	8	13	14	15	13	20	14	8	4	129	100
Moniezia spp. ...	1	1	2	—	—	2	1	6	5	5	2	3	28	21·7
Moniezia (young) ...	5	2	1	1	—	1	3	6	6	6	3	2	36	27·9
Monodontus trigonocephalus	6	2	10	7	11	13	4	6	4	3	3	4	72	55·8
Nematodirus filicollis	6	1	3	2	—	2	—	6	12	9	7	4	52	40·3
Trichostrongylus vitrinus	7	1	1	2	—	1	—	2	6	6	2	2	30	23·2
Cooperia oncophora	—	—	—	—	—	—	1	4	5	5	2	—	17	13·2
Cooperia curticei ...	—	1	—	1	3	—	—	—	2	—	1	—	8	6·1
Capillaria longipes...	—	—	—	1	—	1	—	—	—	1	—	—	3	—
Ascaris ovis ...	—	—	—	—	—	—	—	2	—	—	—	—	2	—
Oesophagostomum venulosum ...	7	1	9	4	3	7	—	5	6	8	2	1	53	41·0
Chabertia ovina ...	—	—	—	—	—	—	—	1	2	1	—	1	5	—
Trichuris ovis ...	7	1	8	3	1	2	—	4	12	12	6	4	60	46·4
Ostertagia circumcincta	2	1	3	—	—	2	—	6	8	9	4	3	38	29·3
Hæmonchus contortus	2	1	1	—	—	—	—	—	2	3	2	—	11	8·5
Ostertagia trifurcata	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dictyocaulus filaria	—	—	—	—	—	—	—	—	4	3	2	—	9	7·0
Muellerius capillaris	—	—	—	1	—	—	—	3	3	—	2	—	9	7·0
Protostrongylus rufescens ...	—	—	2	—	—	—	—	—	—	—	—	1	3	—
Cysticercus tenuicollis	—	—	—	1	2	1	1	1	—	1	—	—	7	5·3
Echinococcus ...	—	—	—	—	—	—	—	3	—	—	—	—	3	—

TABLE D.

Showing the Variations of Helminthic Infection in Lambs and Sheep.

	Spring Lambs.			Other Lambs.			Spring Sheep.			Other Sheep.		
Aeron District.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.
Moniezia spp.	76	34·2	3	111	72·1	—	21	100	—	160	87·5	—
Moniezia (young)	76	31·6	4	111	47·7	1	21	95·2	—	160	89·4	—
Monodontus trigonocephalus ...	76	92·2	—	111	40·5	1	21	19·0	3	160	12·5	10
Nematodirus filicollis	76	21·1	10—20	111	46·8	1	21	85·7	—	160	65·6	—
Trichostrongylus vitrinus ...	76	64·5	—	111	52·3	—	21	95·2	—	160	79·4	—
Cooperia oncophora	76	42·1	2	111	71·2	—	21	100	—	160	98·1	—
Œsophagostomum venulosum ...	76	80·3	—	111	28·8	5	21	90·5	—	160	57·5	—
Trichuris ovis	76	34·2	2	111	12·6	9	21	81·0	—	160	46·3	1
Ostertagia circumcincta	76	27·6	20+	111	55·9	—	21	95·2	—	160	92·5	—
Hæmonchus contortus	76	80·3	—	111	74·7	—	21	100	—	160	97·5	—

	Spring Lambs.			Other Lambs.			Spring Sheep.			Other Sheep.		
Valleys District.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.
Moniezia spp.	29	27·6	3	40	65·0	—	16	100	—	86	90·7	—
Moniezia (young)	29	34·5	2	40	42·5	1	16	100	—	86	93·0	—
Monodontus trigonocephalus ...	29	96·0	—	40	55·0	—	16	6·3	8	86	18·5	6
Nematodirus filicollis	29	34·5	5	40	35·0	10—20	16	100	—	86	82·6	—
Trichostrongylus vitrinus ...	29	75·9	—	40	35·0	4	16	100	—	86	84·9	—
Cooperia oncophora	29	44·8	1	40	77·5	—	16	100	—	86	96·5	—
Œsophagostomum venulosum ...	29	75·9	—	40	42·5	2	16	81·3	—	86	55·8	—
Trichuris ovis	29	34·5	3	40	15·0	10—20	16	62·5	—	86	45·4	2
Ostertagia circumcincta	29	13·8	20+	40	35·0	6	16	93·8	—	86	80·3	—
Hæmonchus contortus	29	89·7	—	40	65·0	—	16	93·8	—	86	97·7	—

	Spring Lambs.			Other Lambs.			Spring Sheep.			Other Sheep.		
Borth District.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.	Number of Observations.	Percentage free from Parasites.	Median Number of Parasites.
Moniezia spp.	31	61·3	—	30	66·6	—	17	100	—	51	96·1	—
Moniezia (young)	31	51·6	—	30	43·3	1	17	100	—	51	90·2	—
Monodontus trigonocephalus ...	31	93·6	—	30	56·7	—	17	23·5	4	51	9·2	10–20
Nematodirus filicollis	31	41·9	20+	30	13·3	20+	17	100	—	51	88·2	—
Trichostrongylus vitrinus... ...	31	67·7	—	30	36·7	4	17	100	—	51	92·2	—
Cooperia oncophora	31	67·7	—	30	73·3	—	17	94·2	—	51	100	—
Œsophagostomum venulosum ...	31	64·5	—	30	46·7	1	17	100	—	51	49·2	1
Trichuris ovis	31	58·1	—	30	3·3	5	17	100	—	51	68·6	—
Ostertagia circumcincta	31	54·9	—	30	36·7	5	17	100	—	51	88·2	—
Hæmonchis contortus	31	93·5	—	30	76·7	—	17	100	—	51	96·1	—

TABLE E.

Showing the Annual Helminthic Infection of the Total Number of Sheep Examined in the Area.

	1924			1925									Total	Per-cent-age.
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.		
Sheep examined ...	20	17	15	11	14	8	10	10	6	20	5	9	145	100
Moniezia spp. ...	10	5	5	1	—	2	4	9	4	6	4	4	54	37·5
Moniezia (young) ...	9	4	4	—	—	1	4	9	4	8	1	5	49	33·8
Bunostomum trigonocephalum ...	16	13	12	8	13	7	3	1	—	5	2	4	84	58
Nematodirus filicollis	12	10	5	1	—	—	5	7	4	12	2	2	60	41·4
Trichostrongylus vitrinus	15	7	10	4	—	—	4	5	3	13	4	4	69	47·6
Cooperia oncophora	1	1	1	—	—	—	—	6	4	7	1	—	21	14·5
Cooperia curticei . .	10	1	1	2	4	1	—	—	—	—	—	1	20	13·8
Capillaria longipes...	2	1	—	1	—	—	1	—	—	—	—	—	5	—
Ascaris ovis ...	—	—	—	—	—	—	—	1	—	—	—	—	1	—
Œsophagostomum venulosum ...	16	12	9	3	3	3	3	2	—	12	5	8	81	55·8
Chabertia ovina ...	8	—	1	—	1	—	2	2	1	7	3	5	30	20·6
Trichuris ovis ...	17	9	10	3	3	1	4	6	4	20	5	7	89	61·4
Ostertagia circumcincta	11	7	5	4	1	[illegible]	5	6	6	18	3	3	71	48·9
Hæmonchus contortus	8	3	1	—	—	—	3	—	—	8	2	1	26	17·9
Ostertagia trifurcata	1	—	1	1	1	1	—	—	—	2	—	—	7	—
Dictyocaulus filaria	2	—	—	3	1	—	—	—	—	—	—	—	6	—
Muellerius capillaris	3	6	1	—	2	1	—	1	—	—	4	6	24	16·7
Protostrongylus rufescens	—	1	—	—	—	—	—	[illegible]	—	—	—	—	3	—
Cysticercus tenuicollis	1	4	2	4	2	1	—	—	1	1	—	—	17	11·7
Echinococcus ...	—	2	—	1	—	2	—	—	1	—	3	1	10	6·8

	1925			1926									Total	Per-cent-age.
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.		
Sheep examined ...	7	12	15	10	25	9	19	34	6	8	—	—	145	100
Moniezia spp. ...	5	3	3	—	2	—	8	16	1	4	—	—	42	28·9
Moniezia (young) ...	4	6	2	1	1	—	10	13	1	2	—	—	40	27·6
Bunostomum trigonocephalum ...	2	11	13	10	18	8	4	10	3	7	—	—	86	59·3
Nematodirus filicollis	2	2	6	—	3	—	8	16	4	2	—	—	43	29·6
Trichostrongylus vitrinus	3	2	6	—	5	—	1	7	1	1	—	—	26	18
Cooperia oncophora	2	1	—	—	—	—	8	[illegible]0	2	3	—	—	36	24·8
Cooperia curticei ...	4	2	1	—	—	1	3	2	2	1	—	—	16	11
Capillaria longipes...	—	—	—	1	1	1	—	1	1	—	—	—	5	—
Ascaris ovis ...	—	—	—	—	—	—	—	1	—	—	—	—	1	—
Œsophagostomum venulosum ...	5	9	11	3	6	—	3	9	1	2	—	—	50	34·5
Chabertia ovina ...	1	2	4	1	—	—	—	—	—	1	—	—	9	—
Trichuris ovis ...	5	9	12	6	10	2	3	16	2	5	—	—	70	48·7
Ostertagia circumcincta	3	4	6	1	2	—	8	[illegible]3	3	3	—	—	53	36·6
Ostertagia trifurcata	1	—	—	—	—	—	—	2	1	—	—	—	4	—
Hæmonchus contortus	2	2	—	—	—	—	3	6	—	—	—	—	13	9
Muellerius capillaris	—	—	—	—	—	—	—	11	2	1	—	—	14	9·7
Protostrongylus rufescens... ...	—	—	1	—	12	—	1	—	—	—	—	—	14	9·7
Cysticercus tenuicollis	—	1	4	2	3	—	4	8	—	4	—	—	21	14·5
Echinococcus ...	—	1	—	3	1	—	—	4	—	1	—	—	10	6·8
Dictyocaulus filaria	2	—	—	—	3	—	1	—	—	1	—	—	7	—

	1926		1927									Total.	Per-cent-age.
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.		
Sheep examined	8	20	26	19	20	18	20	23	16	22	12	204	—
Moniezia spp.	1	6	2	1	1	4	13	3	4	6	5	36	17·6
Moniezia (young)	1	2	4	1	—	4	11	9	10	10	6	58	28·3
Monodontus trigonocephalus	6	19	26	18	10	8	5	4	3	8	11	112	54·9
Nematodirus filicollis ...	6	2	4	—	7	5	14	12	12	16	7	85	41·6
Trichostrongylus vitrinus...	3	1	2	—	1	2	8	8	[illegible]	8	3	42	20·6
Cooperia onchophora ...	1	1	—	—	4	3	12	7	[illegible]	5	2	43	21
Cooperia curticei	2	—	1	—	—	—	—	—	—	4	—	7	3·4
Capillaria longipes ...	—	—	2	—	1	—	—	1	2	—	—	6	2·4
Ascaris ovis	—	—	—	—	—	—	2	—	—	—	—	2	—
Œsophagostomum venulosum	6	17	5	4	4	—	2	8	5	10	8	69	33·3
Chabertia ovina	3	1	—	—	—	—	—	2	—	—	1	7	3·4
Trichuris ovis	8	15	12	8	6	1	13	20	12	17	12	124	60·7
Ostertagia circumcincta ...	4	7	—	—	2	4	10	10	12	12	6	67	32·8
Hæmonchus contortus ...	1	2	—	—	—	—	—	4	1	5	—	13	6·3
Ostertagia trifurcata ...	—	—	—	—	—	—	—	—	—	—	—	—	—
Dictyocaulus filaria ...	2	—	1	—	—	1	1	8	6	5	3	27	13·2
Muellerius capillaris ...	4	—	5	2	—	5	7	5	5	3	—	36	17·6
Protostrongylus rufescens	—	—	—	—	—	—	—	—	2	—	1	3	—
Cysticercus tenuicollis ...	3	2	5	6	2	1	3	—	2	1	—	25	12·2
Echinococcus	—	—	—	—	—	—	—	—	—	—	—	—	—

	1927			1928									Total.	Per-cent-age.
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.		
Sheep examined ...	19	14	12	11	14	22	8	12	15	19	24	—	170	100
Moniezia spp. ...	1	—	1	—	—	1	4	8	5	5	4	—	29	17
Moniezia (young) ...	15	6	—	3	—	1	4	10	5	12	13	—	69	40·6
Monodontus trigonocephalus	18	14	12	11	13	19	6	2	4	—	8	—	107	63
Nematodirus filicollis	13	8	2	—	—	1	—	9	11	17	12	—	73	43
Trichostrongylus vitrinus	18	6	3	—	—	—	—	2	—	3	4	—	36	21·2
Cooperia oncophora	1	—	—	—	1	1	—	4	3	7	5	—	22	13
Cooperia curticei ...	3	—	—	—	—	1	—	—	—	1	2	—	7	4
Capillaria longipes...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ascaris ovis ...	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Œsophagostomum venulosum ...	17	13	12	7	3	7	2	2	2	4	19	—	88	51·8
Chabertia ovina ...	—	—	—	—	—	—	—	—	—	2	1	—	3	—
Trichuris ovis ...	18	13	12	7	3	11	4	6	11	17	24	—	126	74
Ostertagia circumcincta	10	2	—	—	—	1	—	9	7	11	5	—	45	26·5
Hæmonchus contortus	8	4	—	—	—	—	—	—	2	1	7	—	22	13
Ostertagia trifurcata	—	—	—	—	—	—	—	—	—	—	2	—	2	—
Dictyocaulus filaria	3	—	2	—	—	—	—	—	—	5	5	—	15	9
Muellerius capillaris	3	—	6	—	—	2	—	1	—	—	4	—	16	9·4
Protostrongylus rufescens ...	2	—	—	—	—	—	—	—	—	—	—	—	2	—
Cysticercus tenuicollis	1	1	3	1	2	4	—	—	1	—	2	—	15	9
Echinococcus ...	—	1	1	—	—	—	—	—	—	—	—	—	2	—

TABLE F.

SHOWING THE ACTUAL NUMBER OF PARASITES COLLECTED FROM INDIVIDUAL ANIMALS FROM THE *Borth* DISTRICT.

DATE.	Type of Animal.	Moniezia spp.	Moniezia (young).	Monodontus trigonocephalus.	Nematodirus filicollis.	Trichostrongylus vitrinus.	Cooperia oncophora.	Œsophagostomum venulosum.	Trichuris ovis.	Ostertagia circumcincta.	Hæmonchus contortus.
1924.											
Dec. 18th ...	S	—	—	Nil	Nil	—	—	—	—	—	—
,, 19th ...	S	—	—	2	—	—	—	11	14	—	—
,, 19th ...	S	—	—	1	—	+	—	2	23	6	—
1925.											
Jan. 29th ...	S	—	—	27	—	12	—	1	7	—	—
,, 29th ...	S	—	—	—	—	—	—	—	—	—	—
Feb. 2nd ...	S	—	—	15	—	—	—	—	—	—	—
,, 2nd ...	S	—	—	7	—	—	—	—	—	—	—
,, 2nd ...	S	—	—	43	—	—	—	118	—	—	—
,, 16th ...	S	—	—	Nil	Nil	—	—	—	—	—	—
March 13th ...	S	—	—	22	—	—	—	1	—	—	—
,, 23rd ...	S	1	—	108	—	—	—	2	—	57	—
,, 23rd ...	S	2	2	48	—	35	—	3	—	28	—
May 7th ...	L	1	16	—	100	14	18	2	—	36	—
,, 21st ...	L	105	11	—	+	—	—	—	—	1	—
June 3rd ...	L	—	—	Nil	Nil	—	—	—	—	—	—
July 30th ...	L	—	2	—	23	—	—	—	4	+	3
,, 30th ...	L	1	—	—	103	50	3	6	5	23	2
,, 31st ...	L	4	—	—	—	10	—	15	3	80	8
Nov. 10th ...	S	2	2	24	+	12	—	71	20	5	2
,, 20th ...	S	—	2	9	—	—	—	—	—	—	—
1926.											
March 4th ...	S	—	—	67	—	—	—	—	—	—	—
,, 4th ...	S	—	—	47	—	—	—	—	—	—	—
,, 4th ...	S	—	—	21	—	—	—	—	—	—	—
April 14th ...	S	—	—	Nil	Nil	—	—	—	—	—	—
,, 14th ...	S	—	—	Nil	Nil	—	—	—	—	—	—
,, 14th ...	L	—	—	Nil	Nil	—	—	—	—	—	—
,, 21st ...	S	—	—	17	—	—	—	—	—	—	—
,, 28th ...	S	—	—	—	—	—	—	—	—	—	—
,, 28th ...	S	—	—	4	—	—	—	—	—	—	—
,, 29th ...	S	—	—	12	—	—	—	—	—	—	—
,, 29th ...	L	5	2	—	—	—	4	—	—	—	—
,, 29th ...	L	—	1	—	—	—	—	—	—	—	—
,, 29th ...	L	—	1	—	—	—	—	—	—	—	—
May 3rd ...	S	—	—	1	—	—	—	—	—	—	—
,, 3rd ...	S	—	—	1	—	—	—	—	—	—	—
,, 3rd ...	S	—	—	5	—	—	—	—	—	—	—
,, 4th ...	S	—	—	1	—	—	—	—	—	—	—
,, 4th ...	S	—	—	13	—	—	—	—	—	—	—
,, 4th ...	S	—	—	11	—	—	—	—	—	—	—
,, 17th ...	L	10	15	—	+	+	8	3	6	10	—
,, 17th ...	L	14	4	—	+	—	1	1	1	40	—
,, 17th ...	L	—	—	—	—	—	—	3	3	10	—
June 1st ...	S	—	—	11	—	—	—	—	—	—	—
,, 1st ...	S	—	—	1	—	—	—	—	—	—	—
,, 2nd ...	L	—	—	—	+	—	—	—	—	42	—
,, 2nd ...	L	1	3	—	+	1	11	1	2	100	1
,, 2nd ...	L	—	—	—	+	—	2	—	—	50	—
,, 15th ...	L	—	—	3	2	5	—	—	—	—	—
July 13th ...	S	—	—	8	—	—	—	22	—	—	—
,, 13th ...	S	1	—	11	—	—	—	14	18	—	—
,, 13th ...	S	—	—	15	—	—	—	—	—	—	—
Dec. 3rd ...	S	—	—	2	—	—	—	5	23	—	—
,, 3rd ...	S	3	—	4	+	—	—	5	27	2	—
,, 3rd ...	S	1	3	3	+	—	—	1	—	—	—
,, 3rd ...	S	—	—	9	11	—	—	—	—	8	2
1927.											
Jan. 24th ...	S	—	—	22	—	—	—	—	—	—	—
,, 24th ...	S	—	1	13	6	1	—	—	—	—	—
,, 24th ...	S	—	—	14	2	—	—	—	—	—	—
March 9th ...	S	—	—	—	—	—	—	2	2	—	—
,, 9th ...	S	—	—	13	—	—	—	51	—	—	—

Date.	Type of Animal.	Moniezia spp.	Moniezia (young).	Monodontus trigonocephalus.	Nematodirus filicollis.	Trichostrongylus vitrinus.	Cooperia oncophora.	Œsophagostomum venulosum.	Trichuris ovis.	Ostertagia circumcincta.	Hæmonchus contortus.
1927											
Mar. 9th ...	S	—	—	45	—	—	—	—	—	—	—
,, 9th ...	S	—	—	9	—	—	—	—	—	—	—
,, 10th ...	S	—	—	33	—	—	—	3	17	—	—
,, 10th ...	S	—	—	17	40	—	—	—	—	—	—
,, 10th ...	S	—	—	10	2	—	—	3	—	—	—
,, 10th ...	S	—	—	4	—	—	—	—	—	—	—
April 21st ...	S	—	—	38	—	—	—	—	—	—	—
,, 21st ...	S	—	—	Nil	Nil	—	—	—	—	—	—
,, 29th ...	L	—	—	—	—	4	—	—	—	—	—
,, 29th ...	L	—	—	—	—	6	—	—	—	—	—
,, 29th ...	L	—	—	Nil	Nil	—	—	—	—	—	—
May 14th ...	L	87	29	—	80	—	—	40	—	—	—
,, 24th ...	L	10	14	—	24	—	31	—	2	+	—
June 8th ...	S	—	—	8	—	—	—	—	—	—	—
,, 21st ...	L	3	25	—	+	—	—	—	1	—	—
,, 21st ...	L	6	32	—	+	61	—	1	5	+	—
,, 21st ...	L	—	—	—	30	—	6	4	3	—	—
,, 21st ...	L	—	—	—	+	—	—	1	1	—	—
,, 27th ...	L	—	5	—	+	+	20	7	51	+	—
,, 27th ...	L	—	—	—	129	12	9	3	8	+	1
,, 27th ...	L	—	—	—	—	—	—	—	7	16	—
,, 27th ...	L	—	—	—	60	9	—	—	31	40	—
July 4th ...	L	24	32	—	+	12	156	1	13	+	—
,, 4th ...	L	—	7	—	+	11	151	6	6	12	—
,, 4th ...	L	—	1	—	110	—	62	2	5	+	—
,, 4th ...	L	—	2	—	28	8	80	4	2	+	—
Aug. 15th ...	L	—	—	—	+	—	—	—	2	—	—
,, 15th ...	L	3	3	5	69	86	—	—	15	—	—
,, 15th ...	L	—	—	1	+	—	—	—	—	—	—
,, 15th ...	L	—	—	3	+	—	—	—	4	5	—
,, 24th ...	L	—	1	—	36	—	4	—	4	10	—
,, 24th ...	L	—	2	—	51	—	8	22	43	+	3
,, 24th ...	L	1	—	—	80	2	—	10	20	+	23
,, 24th ...	L	—	—	Nil	Nil	—	—	—	—	—	—
Sept. 6th ...	L	3	2	8	12	—	—	—	5	+	—
,, 6th ...	L	4	—	21	80	39	—	9	18	1	—
,, 6th ...	L	2	2	5	8	28	—	—	4	+	—
,, 6th ...	L	—	—	1	5	—	—	—	11	—	—
Oct. 13th ...	L	—	3	2	+	+	—	24	45	—	—
,, 13th ...	L	1	1	13	5	4	—	36	56	—	—
,, 13th ...	L	—	—	1	2	3	—	2	1	—	1
,, 13th ...	L	—	7	—	19	2	—	15	10	—	—
,, 21st ...	L	—	2	2	—	5	—	46	27	2	15
,, 21st ...	L	—	6	5	42	122	—	9	5	—	—
,, 21st ...	L	—	—	6	11	161	—	7	6	2	—
Dec. 15th ...	S	—	—	38	—	—	—	18	5	—	—
,, 15th ...	S	—	—	22	—	—	—	30	4	—	—
,, 15th ...	S	—	—	32	—	—	—	18	12	—	—
,, 15th ...	S	—	—	27	—	—	—	21	9	—	—
1928.											
Jan. 27th ...	S	—	—	108	—	—	—	12	—	—	—
,, 27th ...	S	—	—	11	—	—	—	3	12	—	—
,, 27th ...	S	—	—	28	—	—	—	1	1	—	—
Feb. 7th ...	S	—	—	13	—	—	—	2	—	—	—
,, 7th ...	S	—	—	2	—	—	—	1	—	—	—
,, 21st ...	S	—	—	1	—	—	—	—	—	—	—
,, 21st ...	S	—	—	—	—	—	—	—	—	—	—
,, 21st ...	S	—	—	33	—	—	—	—	—	—	—
,, 24th ...	S	—	—	1	—	—	—	—	—	—	—
,, 24th ...	S	—	—	5	—	—	—	—	11	—	—
,, 24th ...	S	—	—	1	—	—	—	—	—	—	—
,, 24th ...	S	—	—	3	—	—	—	—	—	—	—
June 8th ...	L	10	31	—	14	—	—	—	—	—	—
,, 8th ...	L	—	—	—	—	—	—	—	2	—	—
,, 8th ...	L	—	—	—	—	—	—	—	2	—	—
,, 8th ...	L	5	1	5	—	—	—	—	5	—	—
July 5th ...	L	—	—	—	—	—	—	—	5	120	—
,, 5st ...	L	—	1	—	+	2	20	—	2	—	—
,, 5th ...	L	—	2	—	+	—	—	—	10	84	—
,, 5th ...	L	2	—	—	+	—	—	—	5	84	—

Moniezia spp.—In the preceding tables it may be noted that no data are given concerning the occurrence of the different species of Moniezia. More attention has been paid to the occurrence of the various stages in the growth of Moniezia, for Morgan (1925) found that, as far as his investigation showed, "the parasite is most common in May, there is a distinct drop in percentage infected in mid-summer, with a rise again in August and September." The table below clearly shows that this tapeworm is usually most common in May.

TABLE G.

SHOWING THE SEASONAL INCIDENCE OF TAPEWORMS IN SHEEP.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.
1924–25.												
Number of sheep examined ...	20	17	15	11	14	8	10	10	6	20	5	9
Percentage infected with adult forms	50	29·4	33·3	9	—	25	40	90	66	30	80	44
Percentage infected with young forms	45	23·5	26·6	—	—	12·5	40	90	66	40	20	55
1925–26.												
Number of sheep examined ...	7	12	15	10	25	9	19	34	6	8	—	—
Percentage infected with adult forms	70	25	20	—	8	—	42·1	47	16·6	50	—	—
Percentage infected with young forms	56·2	50	13·3	10	4	—	52·6	38·4	16·6	25	—	—
1926–27.												
Number of sheep examined ...	—	8	20	26	19	20	18	20	23	16	22	12
Percentage infected with adult forms	—	12·5	30	7·3	5·3	5	22·2	65	13	25	27	41
Percentage infected with young forms	—	12·5	10	15·4	5·3	—	22·2	55	39	62·5	45	50
1927–28.												
Number of sheep examined ...	10	14	12	11	14	22	8	12	15	19	24	—
Percentage infected with adult forms	5·3	—	8·3	—	—	4·3	50·0	66·6	33·3	27·0	16·6	—
Percentage infected with young forms	79	42·8	—	27·2	—	4·5	50·0	83·3	33·3	63·1	54·1	—

The number of tapeworms in individual lambs is also generally greater during this month. There is a drop in the percentage infected in June with a rise again from July to October, after which month a general decrease sets in about November to December. From January to March Moniezia are few or absent, but specimens have been collected in every month of the year. It is also evident that Moniezia is a common parasite from May to December and that the young forms (under 4 in. long) occur with somewhat similar fluctuations as the adult (4 in. long and over) forms.

It appears also that these tapeworms grow very rapidly, and after reaching the adult or mature stage remain for a comparatively short period in the intestines. For instance, the percentage of adult forms in November, 1924, is 29·4 and of the young forms 23·5. In December, therefore, one would expect to find about 53 per cent. adult forms, and

not 33·3 per cent. as shown. Similarly the rapid decrease in January and February of 1923 may be explained by the rapid growth and short life of Moniezia within its host.

These fluctuations may possibly be eliminated if more individual hosts were examined for a longer period. Or it may be that the fluctuations are the result of some constitutional changes in the host; it may, perhaps, be influenced by the system of grazing, or, as Morgan (1925) suggests, " a similar periodicity in the occurrence of the intermediate host, assuming that such exist may account for this."

Monodontus trigonocephalus.—This species is usually found to be very common in adult sheep, and particularly numerous from November to May. It does not occur in young lambs before the end of June or the beginning of July. From that time onwards the number increases, the big increase occurring about October. On one or two occasions a single adult specimen has been collected from lambs in May, but this may be considered accidental infection.

This periodicity in *M. trigonocephalus* occurs only in lambs, for it is commonly present in adult sheep throughout the year. It is interesting to note that the parasite is usually absent from, or occurring in very small numbers in the intestines of lambs during April, May, June and July; and that during these months the Trichostrongyle parasites (*Nematodirus filicollis*, *Trichostrongylus vitrinus*, *Cooperia oncophora* and *Cooperia curticei*) are usually present in large numbers. On the other hand, when there are large numbers of *Monodontus*, the Trichostrongyle worms are generally few or absent.

The absence of *M. trigonocephalus* from the small intestines of lambs during April, May, June and July may perhaps be due to the unfavourable physiological conditions of the intestines, or to the influence of the presence of very numerous Trichostrongyle parasites. It is clear also (see Tables A. to F.) that *M. trigonocephalus* runs very closely with *Trichuris ovis* as the most common parasite of the sheep of the whole of this area. It was present in 55·8 per cent. of the sheep from the Borth district; in 64·3 per cent. of those from the Valley district, and in 60·0 per cent. of the sheep from the Aeron district. It infested, on an average for the four years, some 58·9 per cent. of the total number of sheep examined.

Nematodirus filicollis.—On an average this parasite was present in about 38 per cent. of the sheep examined. It was obviously more numerous in the young lambs than in the older sheep, and as the lambs grew older it became less common and numerous, unless the animal was in a bad condition.

Trichostrongylus vitrinus.—Usually this parasite was found to be common and abundant during the same period as *N. filicollis*. Generally found in young lambs it was more rarely met with in old lambs or sheep. It was less common than *N. filicollis* and occurred in about 24-26 per cent. of the total number of sheep examined.

Cooperia oncophora.—Like the Moniezia and the two previous intestinal Trichostrongyles, this parasite was most commonly found in young lambs from May to July or August. It was not so common as either and occurred only in about 18 per cent. of the sheep examined.

Cooperia curticei.—This small parasite was by no means common. It occurred on a few occasions in fairly large numbers, but usually the numbers were very small indeed.

Trichuris ovis.—It may be said of *T. ovis* that it was the most common parasitic worm in the whole area. It was somewhat less common in the Borth district where 46·4 per cent. of the sheep were infected with it, than it was in the Valley and Aeron districts where 62·5 per cent. and 60·0 per cent. respectively harboured the parasite. It was present in young lambs and older sheep alike. On an average it was present in 61·1 per cent. of the total number of sheep examined throughout the period.

Oesophagostomum venulosum.—This parasite was often found with *Trichuris ovis* but was not so uniformly numerous as the latter. It was collected from about 43 per cent. of the animals. In the second and third year of this investigation the percentage of sheep harbouring this parasite was much lower than that of the first and last year.

Chabertia ovina was collected on many occasions but never in large numbers. In 1927 some 200 sheep were examined for this parasite, but only in two cases was it obtained, and only five specimens collected.

Ostertagia circumcincta and O. trifurcata. Of these two species found in the fourth stomach of lambs *O. circumcincta* was by far the more common and numerous. *O. trifurcata* has been identified on 10 occasions only ; it may be more common than is indicated in the tables for the time

taken for the general survey has allowed little opportunity to separate these two species on all occasions. These worms were rarely found in the older lambs and adult sheep and particularly so during January, February and March. Although very large numbers have been collected from individual hosts no cases of deaths caused by these parasites have been reported or traced in any of the districts surveyed.

Hæmonchus contortus.—This fourth-stomach worm was neither so common nor so abundant as *O. circumcincta.* It was collected from April to December, but was most common from July to November. It was seldom found in large numbers in the animals at the slaughter-houses. From two lambs brought to the Municipal slaughter-house on October 11th, 1927, from the Aeron district, as many as 316 and 252 of these parasites were collected. These were the largest numbers obtained. Visits to the farms from which the more heavily infected animals came revealed the probability that the parasite is more common than indicated by the result of the examination of animals at the slaughter-houses, and that it may be the cause of the death of lambs.

In the district of Talybont (about 9 miles north-east of Aberystwyth) there occurs a disease among lambs during July, August and September known to the local farmers as *Pwd.* This particular disease is diagnosed by the presence of an edematous swelling under the lower jaw, dullness, emaciated appearance and the wool harsh and loose. *Pwd* is also used in some districts of Wales for any disease giving rise to the swelling under the lower jaw ; for instance, in the district immediately south of Aberystwyth the term may be used for a stage of the " liver-rot " disease when the swelling occurs under the lower jaw. Although the writer has been unable to obtain a case for post-mortem examination it is believed that this particular *Pwd* is *Parasitic-gastritis* caused by *H. contortus.*

Dictyocaulus filaria was present in the lungs of lambs and sheep of all ages and occurred throughout the year. It was equally prevalent in the three districts.

Muellerius capillaris was the most common parasite of the lungs of sheep. Only in a few cases were the specimens counted owing to the necessity of teasing the parasite out from the lung tissue. Its presence, however, was easily ascertained by greyish proliferations of the lung tissue. Larval forms were found in abundant numbers.

Protostrongylus rufescens was not a very common parasite, but more so than indicated in the appended tables, for cases of infection were observed in lambs and sheep not included in the tables.

Cysticercus tenuicollis was found throughout the year in lambs and adult sheep. It occurred most frequently in the mesentery, but was often found in the liver.

Echinococcus (*hydatid*) cysts were occasionally collected from the lungs of sheep.

Capillaria longipes was not a common parasite, and did not occur in large numbers. It was collected at irregular intervals from the small intestines of lambs and sheep. The largest number obtained from one host was 8 specimens.

Fasciola hepatica, causing " liver-rot " was exceedingly common during September to April. Cases have been observed throughout the year, and reports of death caused by the parasite are frequent during the winter months. There does not seem to have been a very serious outbreak during 1924-28, although deaths have occurred to a fairly considerable number in all three districts.

Cœnurus cerebralis in the brain of sheep was by no means uncommon. Many cysts were collected annually from the slaughter-houses, and *gid* or *sturdy* (*Bendro* in Welsh) has been observed in many instances among flocks at the various farms visited.

Cattle.

The number of cattle examined was 48, all of which were bred within 18 miles of Aberystwyth, and brought from the Aeron district.

The helminthic parasites collected from these animals were few.

Fasciola hepatica was the most common parasite of cattle, but only in a few cases were the numbers, collected from a single liver, very large. It was present in 42 cattle or in 87·5 per cent. of the number examined. Only 12·5 per cent. were entirely free from this " fluke." Not only was a large percentage of the local cattle highly infected but of the total number of cattle brought in from all districts a large percentage suffered from infestations by " fluke." Only two cases of death from this parasite were observed within the period of investigation and these were of two young heifers in the Aeron district. Visits made to various farms, however, showed that a large number of the cattle, particularly the young cattle,

showed symptoms of "liver-rot" disease. Faeces of the suspected individuals were collected and examined microscopically. Fluke-eggs were found to be fairly abundant in the faeces. This high infection of cattle seems to be the result of the ignorance of many farmers concerning the hosts of the "liver-fluke." They do not seem to be aware that cattle also are liable to the "liver-rot" disease. The writer has observed that farmers often remove their sheep from contaminated fields and turn the cattle, a "stronger" stock into them, believing that these animals will not succumb to the disease.

Moniezia spp. were obtained from the small intestines on six occasions.

Capillaria spp. were obtained from the small intestine of one animal.

Monodontus phlebotomum from the large intestine was obtained from 8 cattle.

Dictyocaulus viviparus.—By arrangement with the local Veterinary Surgeon, eggs of this parasite were obtained from the mucus of the windpipe of young cattle at various farms. No adult specimens of this parasite have been collected though the disease "hoose and husk" (*Whéch* or *peswch-ar-loi* in Welsh) caused by it is very common in the district and causes considerable loss (not necessarily death of the animal) to the farmers. Farmers in the Aeron district live in constant dread of this disease during late summer and early autumn.

PIGS.

Of the pigs brought to the Aberystwyth Municipal slaughter-house 62 were examined in the course of this investigation.

Ascaris lumbricoides was a very common parasite of the small intestines. The writer's attention was drawn to two cases of death caused by this worm in two young pigs at a farm 3½ miles from Aberystwyth.

Trichuris suis was a common parasite of the cæcum, but did not usually occur in large numbers.

Oesophagostomum dentatum was not often collected. It occurred in large numbers in the large intestine of one pig only.

Cysticercus tenuicollis was fairly common, but usually found attached to the liver, and rarely on the mesentery.

Fasciola hepatica was obtained from the livers of 8 pigs but in very small numbers.

Metastrongylus elongatus and *M. brevivaginatus* (*Metastrongylus* (*Chœrostrongylus*) *brevivaginatus*, Gedœlst, 1923) parasitic in the bronchioles of the lung were the most common parasites of the pigs examined during this survey. These parasites have been subject to a special study by the writer (Lewis, 1926).

POULTRY.

Walton (1917 and 1924) records the following helminths from poultry in North Wales :—

Raillietina echinobothrida from several fowls in Anglesey, *Hymenolepis lanceolata* from goose, *Davainea cesticillus* obtained once from a fowl which had originally come from Radnorshire, *Heterakis vesicularis* (identified from ova in droppings) and *Syngamus trachealis* which caused losses " here and there, but by no means abundant."

Apart from Walton's work no account of the occurrence of helminths in poultry of Mid-west Wales seems to have been published.

CHICKENS.

Out of 78 chickens examined during this survey only 5 were free from infection by some helminthic parasite.

Heterakis vesicularis was, by far, the most common parasite of the chicken. It usually occurred in abundant numbers in the cæcum. Of the total number of chickens examined 91 per cent. harboured this parasite.

Capillaria spp. present in the small intestines, and cæcum, have been collected from six chickens. They were not common, nor did they occur in large numbers, the highest number was 15 in one chicken. No identification of the species has been attempted.

i/ *Ascarid~~e~~a lineata* was obtained from the small intestines on two occasions. It has once been observed in the excreta of a chicken.

Davainea proglottina seemed to be a rare parasite in this district. Numerous specimens were collected from the small intestines of two chickens.

Syngamus trachea in the windpipes of young chickens of 6 to 8 weeks old was exceptionally common and caused serious losses to poultry-keepers in the district. The writer has received cases of the disease from Carmarthenshire, Pembrokeshire and Cardiganshire.

Ducks.

Forty-two ducks were examined.

Echinocotyle rosseteri occurring in the small intestines was fairly common. Specimens collected were often in a state of disintegration owing to the " high " condition of the host when examined.

Porrocoecum crassum occurred fairly frequently, but usually in small numbers. As many as 72 have been obtained from the small intestine of one duck.

Polymorphus minutus was obtained from the small intestine on one occasion only. The parasites collected were 83 in number, and these had so affected the intestinal wall that it was raised externally into numerous yellow tubercles.

Capillaria anatis seemed to be very rare. Specimens were collected in small numbers from the small intestines of two ducks.

Geese.

Very few helminths were collected from the 10 geese examined.

Heterakis dispar was collected, in small numbers, from the cæcum of one goose.

Capillaria spp.—A species, not yet identified, was collected from the small intestines of three geese, and one female from the œsophagus of another goose.

Pheasant.

Fifteen pheasants were examined for helminthic parasites, but a greater number of the windpipes were examined for *Syngamus trachea.*

Syngamus trachea was a very common parasite of young pheasants and caused much loss to the local pheasant breeders. Adult pheasants also harbour *S. trachea* and act as carriers of the disease caused by it.

Heterakis vesicularis was commonly found in the cæcum.

Capillaria spp. have been collected from four pheasants. The species has not been identified.

Guinea-Fowl.

Only 4 guinea-fowls were obtained for examination.

Heterakis brevispiculum was present in the cæca of two birds.

Capillaria spp. was collected from the small intestine of one bird.

TURKEYS AND BANTAMS.

These birds were only examined for *Syngamus trachea.* The windpipes only were sent by various farmers so that the writer could study the morphology of the parasite.

The writer expresses his gratitude to Prof. R. T. Leiper, F.R.S., for guidance and advice throughout the period of investigation ; to Prof. R. D. Laurie for valuable help and criticism and to Prof. M. Greenwood, F.R.S., for helpful suggestions concerning the arrangement of the statistical data. Also to Messrs. T. H. Edwards, H. Hughes and Jones Bros., Butchers at Aberystwyth, for their willingness at all times to place at the writer's disposal all available material and information regarding animals slaughtered at the slaughter-houses.

REFERENCES.

LEWIS, E. A., 1926.—"Observations on the Incidence of *Metastrongylus brevivaginatus* and *Metastrongylus elongatus* in Pigs in Central Wales." *Journal of Helminthology*. Vol. iv, No. 3, pp. 123-126.

MORGAN, D. O., 1924.—"A survey of Helminthic Parasites of Domestic Animals in the Aberystwyth Area of Wales." *Journal of Helminthology*, vol. ii, No. 2, pp. 89-94.

MORGAN, D. O., 1925.—"Notes on the Helminthic Parasites of Domestic Animals in the Aberystwyth Area of Wales." *Journal of Helminthology*. vol. iii, No. 3-4, pp. 167-172.

WALTON, C. L., 1917.—"Some results of a Survey of the Agricultural Zoology of the Aberystwyth Area." *Parasitology*, vol. x., No. 2, p. 215. (W.L. 16035.)

WALTON, C. L., 1924.—"Preliminary Report on the Agricultural Zoology of North Wales." Report issued by the Department of Agriculture, University College of Wales, Bangor.

Journal of Helminthology, Vol. VIII., *No.* 1, *February*, 1930, *pp.* 19-48.

On the Bionomics of *Heterodera schachtii* on Potatoes, with Special Reference to the Influence of Mustard on the Escape of the Larvæ from the Cysts.

By Marjorie J. Triffitt, M.Sc.

(*Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

A preliminary publication recording the discovery of mustard as a factor influencing the. cyst formation of *Heterodera schachtii*, and describing various experiments to demonstrate the effects of this factor, has already been made. Mustard seedlings grown to a height of about three inches and then broken down in soil in which a potato plant was immediately thereafter grown, were found to exert some influence on the development of the nematode, which partially inhibited the formation of cysts on the roots of the potato plant. The reduction in the number of cysts developed on the roots of a plant so treated, as compared with a plant grown in infected soil without mustard, was found to average over 60 per cent.

Some investigations into the means by which this reduction was effected were also described. Other organic matter was substituted for mustard in similar experiments, but was found to produce no reduction in the number of cysts on the roots of the host plant. It was therefore concluded that the inhibition was not due to the mere increase in the organic content of the soil produced by the introduction of the mustard seedlings. Both roots and shoots of mustard were found to check cyst formation of the nematode, but the roots effected a much greater reduction than the shoots.

In addition to the factor introduced by the broken-up seedlings in the soil, a further diminution of nematode attack was found to take place when the mustard was grown in the same soil in which the potato

was subsequently planted. It thus became evident, that the growing roots produced some effect on the soil such as would occur if some chemical substance were excreted by them during growth.

The work of the Duke of Bedford and Spencer Pickering (1911), and the findings of Baunacke (1922), Rensch (1922) and Nebel (1926), leave little doubt that some substance is given off from growing roots. The Duke of Bedford's and Pickering's work shows that this substance excreted from certain species of plants may exert a deleterious influence on the growth of other species, while Baunacke, Rensch and Nebel have proved that a substance given off from the roots of the host plant stimulates the larvæ of *H. schachtii* to hatch. There is further evidence that the root excretions of plant species differ from one another, and that these differences are detected by the nematode and enable it to select the roots of the preferred host plant, even though they be surrounded by those of other species.

Investigations into the effects of these root excretions on a strain of *H. schachtii* highly specialised on the potato, and experiments to disclose the nature and means of functioning of the inhibitory factor present in mustard, are described below.

Further Pot Experiments with Mustard.

An experiment to demonstrate the effect of the proximity of growing mustard roots on a potato growing in soil heavily infected with *H. schachtii* has already been described. Two potato plants surrounded by growing mustard seedlings were grown in six-inch plant pots. These showed an average cyst reduction of 94·3 per cent as compared with a control plant grown without mustard. As has been pointed out in the original description of this experiment, the comparatively poor root development of the potato plants grown with mustard causes these cyst counts to give a disproportionate idea of the actual intensity of infection.

As a means of obtaining a more accurate estimation of the effect of the excretions of growing mustard roots on cyst development of *H. schachtii* the following experiment was set up.

Two tubers were planted in pots of infected Lincolnshire soil on the same date. One pot, the control, was watered in the ordinary way, the other was supplied only with water in which mustard seedlings

had grown. At the same time another tuber was planted in a pot of clean, light, uninfected soil, so that the growth and development of the two experimental plants might be compared with the normal. Throughout the period of growth the leaves of the plant watered with mustard root excretions were pale in colour, the shoots exceeded in length those of the other two plants, and the plant as a whole had a somewhat etiolated appearance. About seven weeks after this experiment was set up, the lower leaves of this plant began to yellow and die off. The plant grown in infected soil and watered in the usual way, remained throughout indistinguishable from the plant in clean soil. Seventy days after planting, when all three plants showed signs of dying down, the pots were turned out and the root systems examined. Root development in the two infected plants was approximately equal. Cyst counts were made and it was found that, whereas 624 cysts were present on the roots of the untreated plant, only 35 cysts were found on the roots of the plant treated with mustard root excretions. That is, a reduction of approximately 94·3 per cent. of the cyst count on the control plant had resulted from the mustard treatment. This experiment shows therefore that when a sufficient quantity of mustard root excretion is applied during the growth period of the potato plant, cyst formation on the roots of the latter may be almost completely inhibited. Whether the etiolation noted in the plant treated with mustard root excretion was due to a toxic reaction of the latter or to some other cause remains at present unknown, and further experiments are being carried out to determine this point.

Should this prove to be the case, the value of mustard as a possible method of control might be greatly diminished. It was therefore desirable to test the effects, both on the eelworm and on the potato plant, of a single heavy application of mustard excretion to soil, immediately previous to planting the potato set. Accordingly two pots of infected soil were thoroughly soaked in water containing mustard root excretions. A potato set was then planted in each pot and two other pots of infected soil treated with water only were planted at the same time as controls. The cysts on the roots of these plants were counted 58 days after planting. The cyst counts for the plants treated with mustard root excretion were found to be 814 and 781, while for those which had not received mustard root excretions the counts were 1,581 and 1,395. The average reduction

in the cyst count of the plants treated with mustard as compared with the controls was, therefore, 53·56 per cent. Constant observations on the plants during growth failed to reveal any differences in appearance of the shoots, and the root development was found to be approximately equal in all cases. It was therefore concluded that the plants had, in this instance, received no appreciable injury from the mustard root excretions.

Factors Influencing the Hatching of Larvæ from the Cysts.

Experiments were conducted to determine whether the Lincolnshire strain of *H. schachtii*, highly specialised on the potato, showed the same reactions to certain stimuli causing hatching out of the larvæ as have been recorded for strains parasitic on beet.

As a preliminary test, three hundred cysts were placed in each of two small petri-dishes A and B, containing tap water, and tap water which had been run through a pot of soil containing a growing potato plant, respectively. The petri-dishes were kept at a temperature of 25° C. and were examined for larvæ every forty-eight hours. After four days a considerable number of larvæ had escaped from the cysts in petri-dish B, while only very few were present in the water in petri-dish A. Throughout the fourteen days during which these cysts were kept under observation, larvæ continued to be liberated from the cysts in petri-dish B. The rate of escape, however, showed a marked decrease towards the end of this period ; this was probably due to chemical changes brought about in the solution by algæ and bacterial growths which appeared in abundance in this solution. In petri-dish A, containing tap water only, a few larvæ continued to escape throughout the period, but in numbers negligible as compared with those hatched in petri-dish B.

Although the few larvæ hatched in pure tap water remained alive and apparently healthy in this medium for a considerable period, it seemed possible that the slow rate of hatching might be due to some injury to the eggs within the cysts caused by abnormal osmotic pressure The water in petri-dish A was therefore replaced by water which had been run through a pot of soil containing a growing potato plant. That no material damage had been done to the eggs was shown by the fact that after only twenty-four hours, larvæ began to escape from these

cysts in numbers. As a further test of the sensitivity of eggs of *H. schachtii* to slight osmotic changes, samples of one hundred cysts were soaked in distilled water, tap water and water culture solution for fourteen days. During this time very few larvæ escaped from each cyst sample, but on the replacement of these solutions by leachings from a potted potato plant, the counts of escaped larvæ immediately showed a very considerable increase, which was maintained over a further period of ten days during which the cysts were kept under observation.

These results differ strikingly from the results of Rensch's observations on a strain of *H. schachtii* specialised on Beet. Rensch found that, given suitable conditions of temperature, larvæ of the Beet strain hatched very freely for a short period in water. After a few days the rate of hatching dropped gradually to a very low figure, but could be increased by the addition of " stimulant " obtained from the roots of beet plants. Repeated experiments with the Lincolnshire potato strain have in every case shown that only a very few larvæ emerge from the cysts in the absence of the specific stimulus exerted by the potato roots. During certain months of the summer, larvæ hatch more freely in water than at other times, but this phenomenon is dealt with below.

Since the solution which had been found to produce hatching of the larvæ was obtained in such a manner as to include dilute solutions of mineral and organic substances present in soil, as well as excretions of potato roots, it remained uncertain from which of these sources the stimulant was derived. As a means of separating these two constituents of the solution, the following method was adopted. Fifty grams of soil were taken from an area in a wood, where the soil was rich in organic matter but surface vegetation was very scanty. One hundred c.c. of water was added to this soil and left for forty-eight hours. It was then filtered off. To obtain the excretions of the potato roots, seeds were germinated on muslin stretched over the lower half of a large petri-dish containing distilled water. This was kept in a germination chamber composed of the inverted upper half of a petri-dish containing a little distilled water, covered over with another half petri-dish. The seeds germinated readily and the roots of the seedlings passed through the muslin into the water, which could be drawn off by a fine pipette Mustard seeds, beet seeds and grass seeds were germinated in the same manner to obtain the root excretions of the seedlings.

Another series of experiments was then set up as follows. Three hundred cysts were placed in each of five small petri-dishes containing the following solutions :—

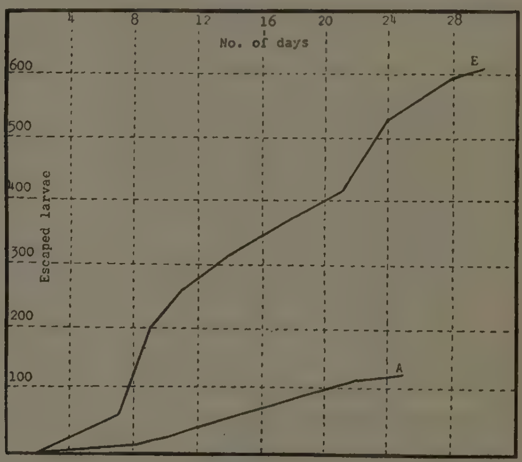
(A) 5 c.c. distilled water.

(B) 5 c.c. soil extract.

(C) 5 c.c. dilute potato excretion water (three drops water from potato germination chamber in 25 c.c. distilled water).

(D) 5 c.c.s. pure potato root excretion water.

(E) 5 c.c.s. pure mustard root excretion water.



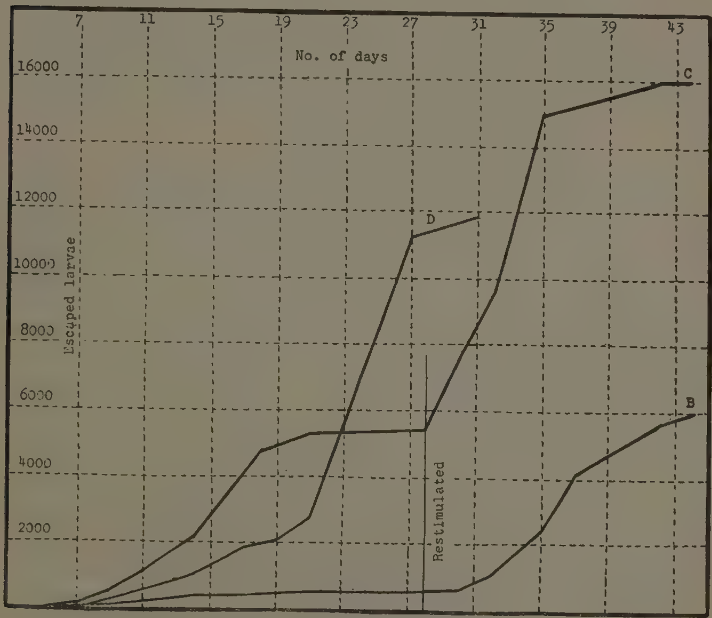
Graph 1.

The complete ranges of figures obtained by the counts from these five experiments are not tabulated, but the results are shown in Graph 1 for Experiments A and E, and Graph 2 for Experiments B, C, D.

The petri-dishes were kept in an incubator at 25° C. and the escaped larvæ were removed at intervals of two to four days, counted, and the dishes filled up with the solutions. Very few larvæ escaped from the cysts in distilled water, the rate of hatching rising from one larva in forty-eight hours to 9 larvæ in twenty-four hours on the fourteenth

day, and then diminishing gradually to 3 larvæ in twenty-four hours on the twenty-fifth day.

In soil extract the larvæ hatched more freely. On the fifth day, 9 larvæ escaped, and during the following forty-eight hours 96 larvæ



Graph 2.

were liberated. Hatching continued at about this rate until the fourteenth day, when it gradually diminished to about 5 larvæ per twenty-four hours on the twenty-fifth day. In the third petri-dish, containing dilute potato root excretion in distilled water the rate of hatching rose from 5 larvæ per twenty-four hours on the fifth day to 60 larvæ per twenty-four hours on the seventh, 192 larvæ on the ninth, 515 larvæ on the fourteenth day, and 662 larvæ on the sixteenth. It then dropped to 17 larvæ per twenty-four hours on the twenty-fifth day. In the fourth petri-dish which contained water

in which potato seedlings had grown, used without dilution, the rate of hatching, though high, was very irregular. It rose per twenty-four hours from 13 larvæ on the fourth day to 212 larvæ on the seventh, sank to 80 on the ninth, rising from the ninth to the fourteenth, then sinking again and rising to 1,408 on the twenty-first and sinking irregularly to 302 on the thirtieth. In diluted mustard excretion water the rate of hatching rose to 71 larvæ per twenty-four hours on the ninth day, dropped to 10 larvæ per twenty-four hours on the sixteenth day, and rose again to 38 larvæ on the twenty-fourth day, after which it sank to 7 larvæ per twenty-four hours.

Since it has been established by Fuchs (1911) that only a percentage of the eggs present within the cysts are liberated in a single year, and that this percentage shows great variation in different individuals, it remained to be proved that the varying number of larvæ that escaped in the tests described above, were really attributable to the intensity of the stimuli administered, and did not merely represent the varying percentages of eggs which had reached maturity in the various cyst samples under observation. After twenty-eight days, therefore, when the daily rate of hatching had sunk to a low figure, two of the solutions, those in petri-dishes B and C, were changed. Soil extract in petri-dish B was replaced by dilute potato-root excretion (3 drops to 25 c.c. distilled water), and dilute potato root excretion in petri-dish C was replaced by undiluted potato root excretion. The immediate result in each case was a marked increase in the rate of hatching, up to 866 larvæ per twenty-four hours after twelve days in petri-dish B, and 1,779 larvæ after ten days in petri-dish C, after which the numbers again dropped. It was thus clearly demonstrated that the escape of the larvæ from the cysts was a definite reaction to the stimulant properties of the media in which they were placed, and the variations in the number of escaped larvæ were not solely due to the varying percentages of mature eggs present in the different samples of cysts.

It should be noted that a possible error was introduced into these results in the following manner. During the counting and removal of the escaped larvæ, the surrounding medium was also necessarily removed, and this was replaced by a similar fresh solution on each occasion. The

root excretions were withdrawn from the germination chambers containing the growing seedlings, and the loss of water thus occasioned, as well as the loss caused by the imbibition of the seedlings was made good from time to time by the addition of distilled water. Thus the concentration of root excretions used must have varied widely, for besides its gradual increase of concentration caused by the presence of the seedlings, it was diluted at irregular intervals by the filling up of the germination chambers. Later work has shown that these variations were unlikely to be sufficient materially to affect the counts.

Since the seedlings used in preparing for these experiments were grown with their roots in distilled water, it was thought that substances other than those given off from healthy roots might have been drawn from them osmotically and that these might influence the hatching of the larvæ. Accordingly, seedlings of potatoes were grown in a similar manner but with a standard water-culture solution instead of distilled water. Three hundred cysts were subjected to the resultant solution and counts of the escaped larvæ were made as before. For about ten days larvæ hatched from these cysts at approximately the same rate as they had hatched in the previous instance when distilled water had been used, but after this period algal and fungoid growths appeared amongst the cysts in such quantities as to make counting difficult and possibly also, to check the rate of hatching. This result led to the conclusion that any substance that might be extracted from the roots of seedlings by the agency of distilled water, other than those substances which were normally excreted by growing roots did not induce any appreciable effects on the hatching of the larvæ.

Another possibility which suggested itself was that any chemical substances in solution in the water in which seedlings had been grown, were present, not owing to the giving off of specific products excreted by the roots, but as a result of the decomposition of root-cap elements and root hairs which were shed into the water. In order to test whether this were the case, or whether true root-excretion products were involved, five seedlings which had been grown for 28 days in distilled water only, were washed thoroughly and their roots were subjected to gentle brushing with a fine camel-hair brush; they were then left in 5 c.c. of distilled water for forty-five minutes. On the removal of the seedlings the water was filtered and sixty cysts were added to it. As a control

measure sixty cysts were also placed in distilled water. Six days later the escaped larvæ in each of these solutions were counted. From the control cysts 20 larvæ only had escaped while 125 were present in the water which had contained the seedlings. It was therefore concluded that some substance had been given off from the roots of the potato seedlings which had stimulated the larvæ to hatch, and that this substance was not merely the decomposition products of decaying root elements.

From the above experiment it appeared that very dilute concentrations of the excretions of roots were sufficient to stimulate the larvæ to escape from the cysts. Experiments were therefore set up to determine, if possible, the minimum and optimum concentrations of secretion for producing this effect. Since the concentration of the substance within the germination chamber remained unknown, and, owing to the lack of knowledge as to its nature it could not be estimated, the following method was adopted. Six petri-dishes each containing 5 c.c. of distilled water were prepared and numbered. Into I, one seedling was placed for half an hour. In II, one seedling was placed for one hour. In III, two seedlings were placed for one hour, and in IV and V, four and eight seedlings were placed respectively, in each case for one hour. Petri-dish VI contained distilled water only as a control. Fifty cysts were placed in each petri-dish ; the escaped larvæ were counted at intervals and the petri-dishes were filled up with solutions obtained in the manner described above. The results of the counts are given in the following table :—

—	1 seedling ½ hour. I	1 seedling 1 hour. II	2 seedlings 1 hour. III	4 seedlings 1 hour. IV	8 seedlings 1 hour. V	Control. VI
4th day	2	24	0	26	85	2
5th day	7	93	3	195	500	8
6th day	43	116	14	607	770	1
8th day	481	320	51	830	690	6
10th day	532	250	70	910	260	2
Total ...	1,065	803	138	2,568	2,305	19

Since all the seedlings had been germinated for the same length of time and their root development was approximately equal (seedlings with

roots of from 35 to 40 mm. in total length were chosen for this experiment) and, further, the cysts were subjected to uniform conditions of temperature and humidity, the striking discrepancy between the number of larvæ hatched in petri-dishes I and III can only be attributed to inherent differences of the cyst samples, probably including the difference in the number of eggs which had reached maturity. It was concluded that fifty cysts was an insufficient number for carrying out experiments of this type. In later work, where larger numbers of cysts have been used, the same error has been found to occur, but this is referred to below. Three other samples of fifty cysts each were placed in more concentrated solutions of root excretions obtained from the germination chambers, and after ten days, counts of the free larvæ were made. In only one instance did the count exceed the maximum number escaped in the former experiment, 2,790 larvæ being freed in this case.

Two conclusions may therefore be drawn from this series of experiments. Firstly, from experiment I, that extremely weak concentrations of the substance excreted by the potato roots are sufficient to stimulate the larvæ to hatch, and secondly, that a solution equal to the strength given off from four seedlings in one hour into 5 c.c. of distilled water, causes the larvæ to hatch in as great an abundance as do stronger concentrations.

The seedlings grown in distilled water for the purpose of obtaining root excretions were found to give off these substances for a limited period only. For a period of six to eight weeks the shoots remained healthy-looking, after which yellowing began and the plants died out. After the sixth week of growth it was found that, if the water in the germination chamber was entirely replaced, it later failed to supply the stimulant necessary to cause hatching when applied to the cysts. A similar condition was found to occur in potted plants grown from tubers. After the growing period, when yellowing of the shoots began, preparatory to dying down, water run through the soil failed to stimulate the larvæ. It was therefore concluded that the substance or substances to which the larvæ reacted were only given off by growing roots.

In order to determine whether the whole of the root system was involved, or whether the stimulant was excreted by the growing point only, another series of experiments was set up. Three healthy seedlings

were each placed in a petri-dish containing 5 c.c. of distilled water in which the whole root was submerged for one and a half hours. Three seedlings with the six terminal millimeters of all roots removed were similarly treated, and the root-tips were immediately placed in another petri-dish with an equal quantity of distilled water, from which they were removed one and a half hours later. One hundred cysts were then placed in each of these petri-dishes, and in two containing distilled water only as controls. Six days later counts were made of the escaped larvæ with the following results :—

From the cysts subjected to the excretions of whole roots 2,420, 3,180 and 5,020 larvæ had escaped, giving an average of 3,540 per 100 cysts. From the cysts subjected to the excretions of roots with their growing points removed, 3,620, 1,980 and 2,440 larvæ had escaped, giving an average figure of 2,680 larvæ per 100 cysts.

In water which had contained the root tips only 3,220 larvæ were counted, while the two controls of distilled water had hatched only twelve and twenty-seven larvæ respectively.

In drawing conclusions from these results, not only the variability in hatching power of different cyst samples, but also the probability of the cut ends of the roots exuding plasma, and the fact that some of the root systems were shorter than others, being curtailed of about one-sixth of their length, must be taken into account. It seems, however, safe to assume that the excretion of chemical products which act as stimulative agents to *H. schachtii* is not confined to the growing points of the roots.

In some of the earlier pot experiments with mustard, it was found that, by breaking up either shoots or roots of the seedlings into infected soil, a diminution of nematode attack on the potato could be produced. When shoots only were used the reduction in attack was less marked than when roots were broken into the soil, and in neither case was so great a decrease in the cyst count apparent as when the seedlings had previously been grown in the same infected soil. Hence it appears that the substance excreted from the roots of the seedlings, or some other substance producing the same effect, was present throughout the whole plant. Rensch, in his earlier experiments, extracted the sap from pulped roots of young sugar beets, and made use of this as

a stimulant to increase the rate of hatching from cysts which had been treated for several days with water. In view of these facts it seemed probable that the substance excreted from potato roots was also present throughout the haulms and leaves, and efforts to extract the substance by simple methods were tried.

Small quantities measured by weight, of root, stem and the first seed leaves of potato seedlings were crushed with pestle and mortar in 10 c.c. of distilled water. The resultant solutions were allowed to stand for three hours before being filtered and added to cysts. The numbers of larvæ freed in these solutions were in every case scanty, though they were rather more numerous in the root-extractions than in those of stem and leaf. Larger quantities, up to 5 grams, of the root, stem, leaf and tuber of potted plants were similarly treated with like results, and it remains to be discovered whether the stimulative substances of potato root excretions are present throughout the plant or are excreted by the roots immediately following their production.

Effects of Boiling and Evaporation on Potato Root Excretion.

Rensch has shown that water containing substances which stimulate the larvæ of a beet strain of the nematode to hatch from the cyst, may be heated to boiling point, and even boiled for a considerable period without destroying the stimulant action of the solution. The effects on potato root excretion of boiling and evaporation were therefore tested.

From a healthy culture of potato seedlings grown for four weeks in distilled water, a quantity of water containing excretions was drawn off. A measured quantity of this solution was brought to boiling point, cooled and placed in a petri-dish (A) with sixty cysts. To another petri-dish (B), containing sixty cysts, solution which had been boiled for five minutes was added, while to a third (C) unboiled solution was added as a control. After eight days petri-dish A contained 113 free larvæ, B contained 2,760 larvæ, and C 250 larvæ.

The high count in B was thought to be a possible result of the concentration of the activating substance consequent on the evaporation of water during the process of boiling. In order to determine whether this were the case another series of experiments was carried out. It

should be noted here that Pyrex glass was used for boiling the excretion in order to avoid contamination from the glass ware.

Nine petri-dishes, each containing sixty cysts, were prepared and were filled with the following solutions :—

1. Water containing excretions obtained from the germination chamber.
2. Water containing excretions heated to boiling point and cooled.
3. Water containing excretions boiled for five minutes.
4. Water containing excretions reduced to half its volume by boiling.
5. Water containing excretions reduced to half its volume and re-diluted to its original volume by the addition of distilled water.
6. Water containing excretions reduced to one-quarter its volume.
7. Water containing excretions reduced to one quarter its volume and re-diluted to its original volume by the addition of distilled water.
8. Distilled water which had been allowed to stand for one hour in a tube in which water containing excretions had been completely evaporated by boiling.
9. Distilled water which had been allowed to stand for one hour in a tube in which distilled water had been completely evaporated by boiling.

Counts of the larvæ freed in these solutions were made periodically and gave the following totals after twenty-one days :—

1. Unboiled solution	1,105	larvæ
2. Heated to boiling point	1,081	,,
3. Boiled for five minutes	294	,,
4. Reduced to half volume	1,006	,,
5. Reduced to half volume and re-diluted	367	,,
6. Reduced to quarter volume	410	,,
7. Reduced to quarter volume and re-diluted ...	857	,,
8. Distilled water added to residue of solution boiled dry	178	,,
9. Distilled water added to tube in which distilled water had been boiled dry	12	,,

The first three of these experiments which were exactly similar to the ones described above gave nevertheless diametrically opposed

results. Thus, where previously low counts had been obtained for unboiled solution and solution heated to boiling point, and a high count for solution which had been boiled for five minutes, only one low count was obtained amongst these three experiments in the second series, and this represented the stimulative power of the solution which had been boiled for five minutes. These experiments failed to establish whether the stimulative efficiency of the solution was impaired by boiling or increased by evaporation as the results were completely masked by the variability in the hatching powers of the cyst samples.

Since a strong concentration of root excretion was used, whereas a weak concentration was sufficient to produce the maximum stimulation of the larvæ, it seemed possible that any reduction in the efficacy of the excretion caused by boiling and evaporation might be masked by the concentration of the solution remaining, even after such reduction, above the optimum.

In an attempt to overcome this difficulty the experiment was repeated with a solution of root excretion (three drops of water from the germination chamber added to 25 c.c.s. of distilled water) of insufficient concentration to produce the maximum possible degree of hatching. Solution three of the previous series was, however, in this case omitted. As was expected, hatching took place more slowly in this series, and the total counts after thirty-four days were as follows :—

1. Solution unboiled	270
2. Solution heated to boiling point	318
3. Solution reduced to half volume	1,171
4. Solution reduced to half and re-diluted to full volume	828
5. Solution reduced to quarter volume	849
6. Solution reduced to quarter and re-diluted to full volume	630
7. Solution completely evaporated and water added ...	306
8. Distilled water completely evaporated and water added	5

This experiment again failed to demonstrate the effect of evaporation and other methods are being employed to obtain the desired result. Two conclusions may however be drawn, first that boiling for fairly prolonged periods does not seriously affect the stimulant properties

of potato root excretion, and secondly that complete evaporation under heat does not entirely destroy the active principle of the excretions. In this connection it seems probable that the comparatively low count shown in this instance in the first series was due to some charring of the residue having taken place.

Reactions of Potato Root Excretions to Influences other than Heat.

Preliminary investigations have been carried out to determine the effect of prolonged evaporation at room temperature on the excretions of potato roots. A quantity of water containing root excretions was withdrawn from a germination chamber containing growing potato seedlings, and was kept at room temperature in a tube plugged with cotton wool to exclude dust. After six months the solution had evaporated to about half its original volume, but remained clear, and free from vegetable growths. Another solution containing excretions, obtained by running water through soil in which a potato plant was growing, was kept for two months in a tube at room temperature. At the expiration of this period the solution contained abundant vegetable growths. The stimulative power of these two solutions was tested by the addition of cysts, and it was found that, while in the sterile solution kept for six months, larvæ hatched freely, the number hatched in the contaminated solution was approximately equal to the number in the control of distilled water. Thus it appears, that whereas under sterile conditions the stimulant remains unchanged for considerable periods, it is quickly broken down and destroyed under non-sterile conditions.

Length of Time Potato Root Excretion remains unchanged in Soil under Field Conditions.

Baermann extractions carried out on soil from a plot of land infected with *H. schachtii* and lying fallow during the summer, showed that free larvæ were present, though not abundant, despite the absence of potato plants. Weeds growing on this land were examined and found to be free from infection. Since for the three previous years potatoes had been cropped on this land, it was thought possible that a small amount of residual root excretion might be present in the soil, in response to which hatching of the larvæ was taking place.

In an attempt to determine whether this were the case, the stimulant action of cold water extracts of soil from this plot was compared with the action of a similar extract of soil in which potatoes had never been grown. The extracts were made by soaking 200 grams of soil in 100 c.c.s. of distilled water for seven days, the water then being filtered off. The first series of extracts was made six weeks after the crop was lifted and the experiment was repeated six months later. Soil from three levels, surface, twelve inches deep and eighteen inches deep was used from the potato plot, while surface soil only was used from the control plot.

In the first series, from one hundred cysts added in each case, 45 larvæ hatched in twenty-four days in extract of surface soil, 75 larvæ in extract of soil twelve inches below the surface, and 56 in extract of soil eighteen inches below the surface, giving an average of 58·6 for the three, while 50 larvæ hatched in the control. In the second experiment the average number hatched by extract of soil from the potato plot was 95·6, while 39 larvæ hatched in the control.

In the following year potatoes were again grown on the experimental plot and soil extracts as before were made three weeks after the crop was lifted. On this occasion the average numbers of larvæ hatched were 25 in extract of soil from the potato plot and 28 in extract of soil from the control plot.

The low counts of escaped larvæ especially noticeable in the first and third series are probably attributable to the beginning of the dormancy period which is discussed below, since these tests were carried out during the early autumn. The second series of extracts which were made in March give a somewhat higher count of larvæ. Since leachings from potatoes grown in pots were found to contain the stimulant given off by the roots into the surrounding soil, it is assumed from the results of these experiments that the excretions produced by the plants during the growing season do not remain unchanged in the soil for a sufficient period to influence the hatching of the larvæ in the following year. This conclusion is confirmed by the observation cited above, that at the end of the growing period, water run through a pot of soil containing a potato plant fails, on its application to cysts, to stimulate the larvæ to hatch from the eggs. The effect of keeping water containing root

excretions under sterile and non-sterile conditions, also referred to above, indicates that the breakdown of the stimulating substance may be brought about by the action of soil bacteria.

The Effects of Mustard Root Excretions on *H. schachtii*.

The effects of mustard seedlings grown and broken into soil infected with *H. schachtii* in inhibiting the formation of cysts on the roots of potatoes subsequently grown in that soil have already been described. Various suggestions as to the method by which the reduction in cyst formation might be brought about have been made, together with the results of experiments to elucidate some of these points. Some of the remaining points have been investigated by laboratory methods and are dealt with below.

It seemed possible that in the first experiments performed with mustard the reduction in cyst formation was due to the early hatching out of the larvæ by the stimulus of the growing mustard, the larvæ then dying out in the absence of a suitable host plant before the potato roots had developed.

Numerous attempts have been made to cause the larvæ of *H. schachtii* to hatch by the application of water containing mustard root excretions to cysts kept under optimum conditions of temperature. Controls of distilled water and soil extract have been used in these experiments, and it has been found that, although the average rate of hatching in water containing mustard root excretions is slightly greater than the average rate in distilled water, it is approximately the same as the rate of hatching in soil extract, and negligible in comparison with the hatching induced by the presence of potato root excretions.

As, however, the mustard root excretion used in these instances was obtained by withdrawal of distilled water from a germination chamber containing mustard seedlings, the combined action of mustard root excretion and soil extract on the eelworm were further tested. Mustard seedlings were germinated in clean soil, and water run through this soil when the plants were about three inches high was applied to 100 cysts. As controls, pure potato and mustard root excretions from the germination chambers, and distilled water, were used. During the time the cysts were under observation only 10 larvæ hatched in the soil

extract combined with mustard root excretion, 7 in pure mustard root excretion, 4 in distilled water, and 511 in pure potato root excretion. It was thus conclusively demonstrated that the presence of growing mustard seedlings in infected soil did not reduce the infection on the potato by inducing the larvæ to hatch while failing to afford them any means of sustenance.

Nebel (1926) has shown that larvæ of a strain attacking beet may remain alive in soil and retain their capacity of attacking the host plant for periods of more than a year. The longevity of free larvæ of the potato strain has not been proved, but it seems reasonable to suppose that this would exceed the short period necessary for the production of roots by a tuber planted immediately after the destruction of the mustard seedlings in the pot experiments.

Another and more probable suggestion as to the method by which reduction of nematode attack, observed in the pot experiments, might have been effected. was that mustard root excretions were toxic to the larvæ within the cyst, causing either death, or inability to escape, and so decreasing the number of larvæ which subsequently hatched in response to the stimulus of the potato.

In order to test this possibility 200 cysts were soaked in distilled water until they had all lost their contained air and sunk to the bottom of the liquid. The distilled water was then drawn off and replaced by a concentrated solution of mustard root excretion. After remaining in mustard root excretion for twenty-four hours, 100 cysts were removed and placed in potato root excretion. Six days later the escaped larvæ were counted and found to number 2,290. The remaining 100 cysts were left in mustard root excretion for fourteen days before being transferred to potato root excretion. After a further period of six days the free larvæ were counted and were found to number 4,020. The difference between these two counts may have been due to the variation in the number of mature eggs in the cyst samples used, or may have been influenced by the longer period of soaking in the second instance, before the potato excretion was added, but the result proves that even prolonged soaking in mustard excretion has no retarding influence on the escape of the larvæ from the cysts.

Since it has been established that the hatching of the larvæ from the

eggs within the cyst takes place as a response to chemical stimulus of a substance given off from the growing roots of the potato plant, whereas the excretions of mustard roots do not have this effect, it may be assumed that the root excretions of these two plants differ in chemical composition. Hence arises the possibility that the root excretions of mustard might neutralise those of the potato and so destroy the stimulant action of the latter.

The effect of combined potato and mustard root excretions on hatching out of the larvæ was therefore tested by laboratory methods. In the first series of hatching experiments, during which accurate counts of escaped larvæ were made, 300 cysts were placed in a petri-dish containing equal quantities of potato and mustard root excretions. Two other experiments which have already been described were set up at the same time and may here be regarded as controls of the experiment with mixed excretions, viz., 300 cysts in pure mustard excretion and 300 cysts in pure potato excretion.

The rate of hatching in this experiment was most irregular but corresponded closely in its rise and fall with the rate of hatching from the cysts in pure undiluted potato excretion. The counts were, however, uniformly higher than in the former case. Thus, the rate of hatching per twenty-four hours rose from 144 on the fourth day to 1,389 on the seventh, then fell to 370 on the ninth, rose again to 697 on the eleventh, fell to 137 on the fourteenth, and rose gradually to 847 on the twenty-first, after which it fell somewhat irregularly to 84 on the thirtieth day. The total count of larvæ which escaped from the cysts during thirty days in the mixed excretions was 13,125. The larvæ freed in pure potato excretion during the same period numbered 11,871, and those in pure mustard excretion numbered 604.

The high rate of hatching and total figure obtained in the experiment where mixed excretions were used to provide the stimulus suggested that the addition of mustard root excretion, instead of checking the stimulative power of the potato excretions, tended rather to increase it.

Another experiment was accordingly set up using equal parts of potato and mustard root excretion. At the same time, the root excretion of another plant known to be non-susceptible to infection by this strain of *H. schachtii*, namely sugar beet, was similarly tested, both with potato

root excretion and alone. In the second trial of a mixture of potato root excretion and mustard root excretion 20,218 larvæ were liberated from 300 cysts in twenty-four days. In pure beet root excretion 324 larvæ were liberated during the same period, and in equal quantities of beet and potato root excretions the total of the counts for twenty-four days was 22,010. This figure, being almost double the count of larvæ escaped from the same number of cysts in pure potato secretion in thirty days seemed too great to be due only to the variation in the number of mature eggs in the cyst samples, more especially as 300 cysts were used in each of these experiments.

To determine whether the combination of root excretion of a susceptible and non-susceptible plant species combined were more efficient in causing the larvæ to hatch than when excretion of a susceptible species was used alone, the following experiments were performed using mustard as the non-susceptible species.

Fifty cysts were placed in each of two petri-dishes A and B containing potato root excretions. After ten days the free larvæ were counted and numbered 234 and 847 respectively. In petri-dish A the potato root excretion was then replaced by equal parts of potato and mustard root excretion, while petri-dish B was filled up with pure potato root excretion. Ten days later counts were again made and 478 larvæ were found in petri-dish A, while only 258 were present in B. This experiment gave therefore a positive result, indicating that the stimulation of the larvæ by potato root excretion was increased by the addition of mustard root excretion, for, previous to the addition of mustard root excretion to petri-dish A, the number of larvæ hatched in A as compared with the number hatched in B was 1 : 3·6, but after the addition of mustard to A, the number hatched rose to a ratio of 1·89 : 1 as compared with B.

A second similar experiment was performed using 100 cysts and continuing the experiment over a longer period. In this experiment, from the cysts in petri-dish A, 2,515 larvæ hatched in pure potato secretion in eleven days, while 3,211 hatched in B. That is A : B = 1 : 1·27. Petri-dish A was then filled up with equal quantities of potato and mustard root excretions, while the pure potato root excretion in B was renewed. Counts of the escaped larvæ in A and B were made at intervals during forty-two days. During this time 7,290 larvæ were hatched in B and

4,560 larvæ were hatched in A. That is, the ratio of the number of larvæ hatched in A as compared with those hatched in B, changed from 1 : 1·27 the ratio when pure potato root excretion formed the stimulus in both, to 1 : 1·59 when mixed excretions formed the stimulus in A. This experiment gave, therefore, a negative result. Two further repetitions of this experiment were made and yielded negative results. It was therefore concluded that no extra stimulus to hatching was occasioned by the admixture of mustard root excretion with potato root excretion, and the differences in the counts of escaped larvæ in the original experiments with mixed excretions must therefore have been due to some other cause.

Although there was no evidence of the stimulant being decreased by mixture of the root excretions in these experiments it seemed possible that, had dilute excretions been used instead of concentrated solutions drawn from the germination chambers, conditions more closely resembling those under which the pot experiments were performed might be produced, and different results be obtained. The following series was therefore set up. One hundred cysts were placed in each of four petri-dishes containing respectively, pure potato root excretion from the germinating chamber; 1 c.c. of potato root excretion in 9 c.c. of distilled water; 1 c.c. of potato root excretion plus 1 c.c. of mustard root excretion in 8 c.c. distilled water; 1 c.c. of potato root excretion in 9 c.c. of mustard root excretion. This series of experiments was carried out three times and counts of the larvæ, which were roughly proportionate in each case, gave the following average results.

1. Pure potato root excretion	322	larvæ
2. 1 c.c. pot root excretion, in 9 c.c. distilled ...	73	,,
3. 1 c.c. potato and 1 c.c. mustard root excretion in 8 c.c. distilled	39	,,
4. 1 c.c. potato in 9 c.c. mustard root excretion ...	17	,,

With regard to this result it must be noted that there was no means of determining the concentration of excretory products in the germination chambers, and therefore it is possible that when equal quantities of the solutions were used, the concentration of mustard root excretion was greater than that of potato root excretion. The conclusion can however, be drawn, that when less than the optimum concentration

of potato root excretion is diluted with a strong concentration of mustard root excretion, the stimulant properties of the former are reduced. It is therefore possible that the small numbers of cysts on the roots of plants treated with mustard was the outcome of this action.

On the Stability of Mustard Root Excretion.

The effects of bacterial action on the root excretions of mustard have not yet been investigated, nor is it known for what period of time this substance remains effective in soil either under natural or experimental conditions. The pot experiments, however, tend to show some evidence that these excretions remain potent under unsterile conditions for somewhat longer periods than the excretions of potato roots. Thus, where mustard root excretions were applied to soil before the tubers were planted, although a short time must have elapsed before the potato roots had developed, on the counts finally being made after fifty-eight days growth, the effects of the presence of active mustard root excretion were abundantly evident. Unfortunately, the roots of the experimental and control plants were not examined for developmental stages of the parasite, and it is possible that nematode attack was merely delayed in the plants treated with mustard. Even had this been the case, however, the delay must have been considerable, since more than the normal time for the completion of the life cycle elapsed before the cyst counts were made.

A further instance in support of the suggestion that the " counter stimulant " present in mustard is not readily destroyed, is shown by the fact that the decay of roots and shoots of the plant broken up in soil, which must necessarily be accompanied by profuse bacterial action, serves to liberate the active principle instead of immediately destroying it.

Observations on the Dormancy Period in *Heterodera schachtii*.

The experiments dealing with the influences of various substances on the emergence of the larvæ of *H. schachtii* from the cysts were begun in the spring of 1928 and continued throughout the summer of that year. It was noticed that during the months of June and July the counts of escaped larvæ were very much higher than they had been previously,

irrespective of the medium in which the cysts were placed and under identical conditions of temperature. During October the counts fell again, although they remained more or less proportionate throughout, varying with the containing medium. Experiments set up in November and December showed such scanty numbers of larvæ that they were abandoned owing to vegetable growths appearing in the cultures before a sufficient number of larvæ had hatched to be of use for comparative purposes. It was thought that contamination of the water in the germination chambers might be the cause of this trouble, and further cultures of seedlings were grown before the tests were resumed. During the following year the same gradual increase in the number of larvæ escaping in all solutions was again noted, followed by a diminution during the autumn months. Again cultures set up in November and December failed to give satisfactory results. On this occasion cysts isolated from infected soil taken from the Institute's field plots were substituted for cysts from Lincolnshire soil, which, kept in an air-dried condition in an unheated shed, had furnished the majority of material previously used. This change in origin of the cysts, however, produced no alteration in the results and the experiments again had to be abandoned. The work was resumed towards the end of January when, although the rate of hatching remained low, sufficient larvæ were liberated to allow comparisons to be made.

This seasonal incidence is responsible for the wide variations which occur in the numbers of larvæ hatched in similar media which are quoted in the foregoing descriptions of experiments. The experiments have been carried out at different periods of the year, but series of tests to investigate any given problem have been set up on the same date, and, with one exception, the results of any given series have been compared only with each other. This exception occurs when the rate of hatching of larvæ in mixed excretions of potato and mustard and potato and beet are compared with results previously obtained for pure potato root excretion and a mixture of potato and mustard root excretion, and although only thirty days had elapsed between the setting up of the two series of experiments, since these took place in the early summer the increased cyst count of the second series must be attributed to the seasonal change.

THE EFFECT OF LACK OF OXYGEN ON THE HATCHING OF THE LARVÆ.

Spencer Pickering has shown in his work on the effect on fruit trees of the root excretions of grass, that whereas the excretions which pass from the roots of the grass, through soil, to the roots of the fruit trees, without being exposed to air, exercise an adverse influence on the growth of the trees, fruit trees watered with the leachings of pots of grass suspended some distance above the soil, showed an increase in growth and seemed to benefit from the grass root excretions. This difference Pickering attributes to the oxidation of the grass root excretions, or some constituent of these, in their passage through the air.

In the hatching experiments carried out in the laboratory, the method of procuring the excretions from seedlings grown on muslin over open petri-dishes gave every opportunity for such oxidation to take place before the excretions were applied to the cysts. Further, in the earliest experiments, when the leachings of pots containing growing potato seedlings were used, these were exposed to air during the hatching period.

In an attempt to test the effect of unoxidised potato root excretions on the hatching of the larvæ, a series of experiments was set up as follows. Potato seedlings were grown in a pot of very fine washed sand. When these seedlings were fairly well grown the pot was watered with distilled water and the leachings were caught in a small tube, containing fifty cysts previously soaked in boiled distilled water, with the minimum possible exposure to air. This tube was then tightly corked to exclude air, and sealed with vaseline. The remainder of the leachings were caught in an open petri-dish containing fifty cysts which had been similarly treated. Seven days later the tube and petri-dish were examined and while the former was found to contain no larvæ, 1,320 larvæ had been freed in the petri-dish. This experiment was repeated, and again no larvæ hatched in the sealed tube while 2,650 hatched in the petri-dish.

In these experiments two factors were obviously involved by the lack of oxygen. Firstly, oxidation of the root excretion was prevented, and this alone might be sufficient to prevent hatching, and secondly, even were this not the case the effect of oxygen deficiency on the larvæ

themselves might prevent response to the stimulus though it were present.

In order to test the effects of lack of oxygen on the hatching power of the larvæ in the presence of oxidised potato root excretions, similar experiments were set up in which root excretions taken from seedlings grown over petri-dishes and exposed to air, were used. Fifty cysts were placed in a small petri-dish containing oxidised excretion and left freely exposed to air. Fifty cysts were placed in a narrow tube, similar to the tubes used in the previous experiments, with oxidised excretion, the tube being left unsealed. Two other samples of fifty cysts each were placed in similar tubes of oxidised excretion which were rendered air-tight by being sealed with vaseline. All the cysts used in these experiments had previously been soaked in boiled distilled water until they lost their contained air and sank to the bottom of the liquid. In order as far as possible to minimise the variation in egg maturity of the samples used, new brown cysts were selected from dried potato roots. Thirteen days after the experiments were set up, the escaped larvæ were counted. In the open petri-dish these were found to number 7,980; in the unsealed tube 7,620, and in the two sealed tubes 53 and 107 respectively. Further, it was noticed that while the larvæ which had been given free access to oxygen were alive and apparently healthy, those in the sealed tubes were all dead. The cysts from the sealed tubes were then removed and placed in a petri-dish with fresh oxidised potato root excretion. Five days later 1,220 larvæ were counted in this petri-dish.

Another sample of fifty cysts was sealed in a tube of oxidised potato root excretion, and in this instance the larvæ were not counted until thirty days had elapsed. About seventy-four larvæ were present in this tube, but all were again dead and the majority were so decomposed as to render counting difficult. On the addition of a fresh solution of excretion and exposure to air, hatching recommenced from these cysts.

From these experiments it becomes evident that the presence of oxygen is a necessary condition for hatching of the larvæ to take place, and that even given suitable conditions of moisture, temperature, and the normal chemical stimulant, hatching is prevented in the absence of

oxygen. Further, since not only was hatching arrested, but the already freed larvæ perished in the sealed tubes when, presumably, the available oxygen was exhausted, it appears that oxygen must be available in order for the larvæ to survive. That the larvæ within the eggs are not influenced, at least for a period of four weeks, by the absence of oxygen in the surrounding medium, is shown by the subsequent hatching of eggs so treated.

A comparison of the results of experiments where oxidised and non-oxidised excretions were used in sealed tubes, gives indeterminate results, for although no larvæ hatched in excretion which had not been exposed to air, and a certain amount of hatching took place in the excretion which had previously been exposed, there was no indication as to whether the controlling factor lay in any change in the chemical composition of the solution, or in the actual presence or absence of oxygen dissolved in the medium and hence available to the larvæ. That the latter was the controlling factor, seems, however, probable.

The Effects of Light and Heavy Soils.

The results of these experiments on the influence of available oxygen over the hatching of larvæ of *H. schachtii* suggest a possible explanation of the fact that this parasite occurs chiefly in regions where the soil is light, sandy, and open in texture. Such soils are, of course, especially suited to potato culture, and the areas affected are therefore those where potatoes are most extensively and intensively propagated. It seems, however, reasonable to suppose that where potatoes are grown fairly constantly in heavy soils, as in gardens and allotments, the parasite would frequently be introduced and become established.

It must not, however, be forgotten that the cultural methods employed in gardens and allotments differ very widely from those adopted by growers on a commercial scale, and the heavy manuring usually given in the former instances might either have an adverse influence on the parasite, or might increase the yield sufficiently to mask any effects of the latter. Nevertheless it seems probable that the oxygen content of soils might exercise some influence on the distribution of the parasite.

Pot experiments with different types of soils were therefore set up as follows. As a preliminary test, two pots, A and B, were filled with heavy clay soil taken from a field on the Institute's Field Station, and

light soil, loam mixed with sand, respectively. To each of these pots 1,200 cysts, isolated from Lincolnshire soil by Morgan's flotation method, were added, a potato set then being planted in each case. Seventy days later the plants were turned out and counts of the number of cysts present on the root systems were made. On the plant in pot A, with heavy soil, only 25 cysts were found, while the plant in pot B, containing light soil had 110 cysts on the roots.

To confirm this result two more potatoes were planted in pots C and D in heavy clay soil taken from an uninfected portion of the experimental plot used for work on *H. schachtii* at Winches Farm. These pots were infected by the addition of cysts isolated from an equal volume of heavily infected Lincolnshire soil. At the same time a potato was planted in pot E, containing infected Lincolnshire soil. After seventy-seven days counts were made of the cysts attached to the roots of these plants. The plants from pots C and D grown in heavy soil bore 15 and 17 cysts respectively on their roots, while the control plant E showed 340 cysts. Taking the average of these figures, and assuming that the potentialities of the cysts used in the experiments and their controls was equal, these results show that in heavy soil only 15·2 per cent. of the larvæ available for carrying on the infection, are successful in doing so.

Reinmuth (1929) carried out similar experiments. Cyst counts were made on plants grown in heavy soil infected with *H. schachtii* to which various proportions of sand had been added. Cyst development was found to be more profuse in soil which had been lightened than in heavy soil. Examination of the cysts formed in these instances, showed that cysts formed on plants in heavy soil tended to be smaller than those developed in lighter soil. Hence, Reinmuth points out, since the egg content is proportional with the size of the cyst, this decrease in cyst dimensions adds another factor limiting the spread of the disease in heavy soils. This observation is of especial interest in that it confirms the present writer's experience with the morphology of the cysts in the field plot at Winches Farm. Here Lincolnshire soil was laid down on heavy land, and, after the cultivation of several crops of potatoes the average size of the cysts was found to be considerably less than the average size of the cysts from the soil originally used to produce the infection.

Reinmuth makes no suggestion as to the factors causing limitation of cyst formation in heavy soil, but to the present writer, lack of aeration appears to be at least one, possibly the principal, factor, though physical conditions which might cause mechanical obstruction to the wanderings of the larvæ and males might also contribute towards this result.

Conclusions.

The conclusions which may be drawn from the results of the experiments recorded above, are, that a chemical substance is given off from the roots of growing potato plants which stimulates the larvæ of *H. schachtii* to hatch from the eggs and emerge from the cysts. The excretion of this stimulant only takes place during the growing season, but its production is not confined to the few terminal millimetres of the root which constitute the region of greatest growth. It remains unknown whether the substance is present throughout the whole plant, but cold water extracts of stem, root, leaf and tuber have given no indication that this is the case. The stimulant substance has been proved to be heat resistant to some extent, and non-volatile, and to remain active in water for long periods under sterile conditions though it is rapidly broken down in the presence of vegetable growths. Extremely dilute solutions of the stimulant are perceived and reacted to by the larvæ.

A substance given off from the roots of mustard plants into infected soil containing a growing potato plant has been shown to check cyst formation of *H. schachtii* on the roots of the latter. There is evidence that this substance is present both in the shoots and roots of the plant, and that it is not so readily destroyed under non-sterile conditions as are the potato root excretions. The inhibitory action of mustard root excretion is due, at least in part, to the fact that when it is present with potato root excretions, and in greater concentration than the latter, the stimulative action of the potato root excretion is so neutralised or masked as to be ineffective. The effects of the chemotactic action of potato and mustard root excretions are not dealt with in the present paper.

That a dormancy period exists in the winter months, during which the larvæ of *H. schachtii* will not hatch even under optimum conditions

of temperature and moisture and in the proximity of potato root excretions, has also been demonstrated.

The presence of oxygen has been found to be necessary for hatching of the larvæ to take place, and to this is attributed the fact that nematode attack is much more severe in light open soils than in soils of a heavier type.

References.

Baunacke, W., 1922.—" Untersuchungen zur Biologie und Bekämpfung des Rübennematoden Heterodera Schachtii Schmidt." *Arb. biol. Aust.* (*Abt.*) *Berl.*, Bd. 11., Heft 3, pp. 185-288. W.L. 1628.

Bedford, Duke of, and Pickering, S. V., 1911.—Thirteenth Report of the Woburn Experimental Fruit Farm.

Fuchs, O., 1911.—" Beiträg zur Biologie der Rübennematoden *Heterodera Schachtii*," *Z. landw. Vchsw. Öst.*, Jahrg. 14, pp. 923-949. W.L. 23488.

Nebel, B., 1926.—" Ein Beiträg zur Physiologie des Rübennematoden Heterodera Schachti vom Standpunkt der Bekämpfung." *Kühn-Archiv.*, Bd. 12, pp. 38-103. W.L. 11941.

Reinmuth, E., 1929.—" Der Kartoffelnematode (Heterodera Schachtii Schm.) Beiträge zur Biologie und Bekämpfung," *Z. PflKrankh.*, Jahrg. 39, Heft. 7, pp. 1-36. W.L. 23540a.

Rensch, B., 1924.—" Eine neue Methode zur Bekämpfung der Rübennematoden," *Mitt. deuts. Landw.Ges.*, Bd. 39, pp. 412-414. W.L. 13900.

Journal of Helminthology, Vol. VIII., *No.* 1, *February,* 1930, *pp.* 49-58.

The Species of *Subulura* Molin in Primates.

By Thomas W. M. Cameron, D.Sc., M.R.C.V.S.

(*Lecturer and Milner Research Fellow in the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

The genus *Subulura* was established in 1860 by Molin for a nematode from *Scops brasiliannus* and at the present day includes some forty species, mostly in birds. Five species have been reported from primates. Three of these are little known and are still only provisionally retained within this genus. These are *S. sarasinorum* (from *Loris*), *S. otolicni* (from *Galago*) and *S. perarmata* (from *Tarsius*). They are discussed below. The remaining species *S. distans* (from *Cercopithecidæ*) and *S. jacchi* (from *Cebidæ* and *Hapalidæ*) are of sufficient importance to merit fuller consideration. The first-mentioned is only incompletely known although the second has been recently re-described by Barreto (1919). During an expedition in 1928 to the Lesser Antilles, I was able to collect a large number of specimens of *Subulura* from the African Monkeys in St. Kitts. These, together with specimens of *S. distans* and *S. jacchi* in the collections of the Department of Helminthology of the London School of Hygiene and Tropical Medicine, provided the material on which this paper is based.

Subulura distans (Rud., 1809) R. & H., 1913.

This species is very imperfectly described although it has been reported from a considerable number of different hosts. Barreto, collating the previous descriptions, gives the following particulars :—

Body rather large, either of uniform width or thinning towards

anterior extremity, spirally rolled. Head roundish, bare. Mouth triangular, with small lips leading directly to a pestle-shaped œsophagus followed by a spherical bulb.

Male. Length, 27 mm. (Duj.); 14·7 to 25·4 mm. (Dies.); 25 mm. (Sch); more slender than female. Tail curved like a hook, ending in a short point. Spicules unequal, very long and curved. Canal bursa little developed. Genital papillæ eleven in number. Elliptical sucker without chitinous ring.

Female. Length, 40 mm. (Duj.); 25·4 to 40·1 mm. (Dies.). Width, 1·5 mm. (Diesing); twice size of male, straight, thread-like; tail with diverging extremity. Eggs round.

The habitat is the Cæcum and Large Intestine.

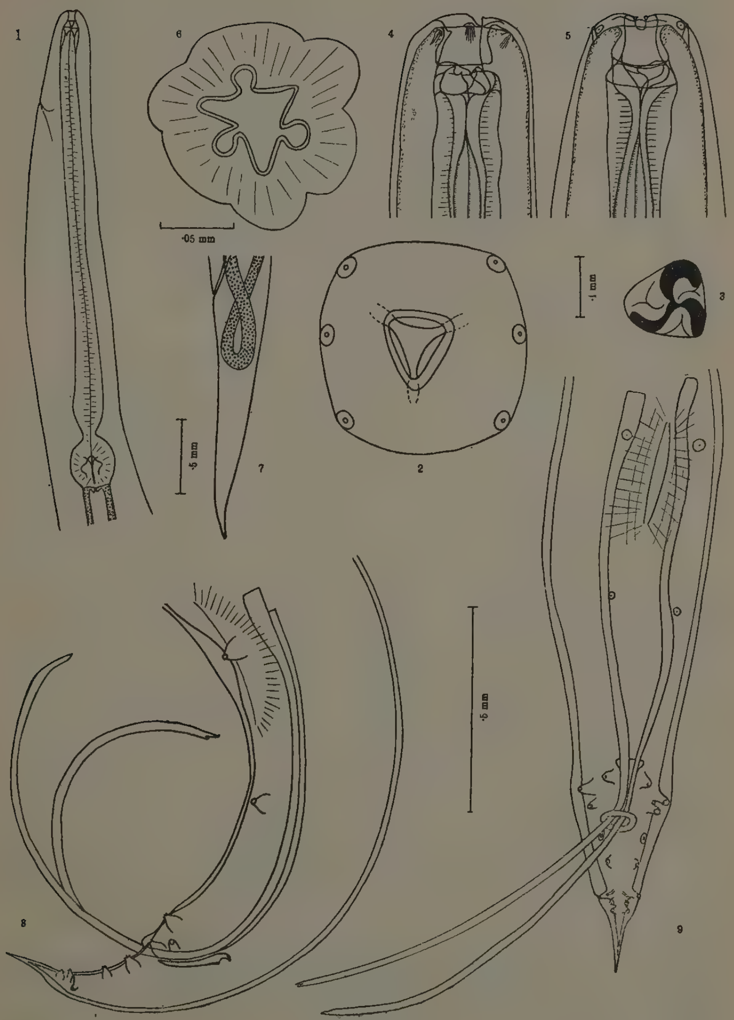
This species has been reported from the following hosts :—*Cercopithecus sabæus* (= *C. callithicus*), *C. cephus*, *C. brazzæ*, *Erythocebus patas*, *Cercocebus fuliginosus*, and *Cercocebus collaris*.

To these hosts there should be added :—*Cercopithecus mona*, *C. grayi* and *C. nictitans*.

The following description of *S. distans* is based on specimens collected from *C. sabæus* from West Africa.

The females vary in length from 23 mm. to 29 mm. with a maximum thickness of 0·95 mm.; the males are 16 mm. to 17 mm. long by 0·6 mm. wide. In both sexes, the body is slightly attenuated anteriorly and the cephalic extremity is terminal (fig. 1). The cuticle is transversely striated. Both cervical papillæ and alæ are absent. The mouth opening is triangular, with the apex of the triangle pointing ventrally (fig. 2). The sides of the triangle are reinforced by sharpened plates, which are formed from a denser cuticle than the rest of the head region. These plates do not meet at the angles of the mouth. On either side of the mouth opening are three papillæ, arranged in a straight line—dorsal, lateral and ventral in position. They are small and sessile. The mouth opening communicates with a large rigid cuticularised buccal cavity which is about as deep as it is wide and is triangular in cross-

Subulura distans from *C. sabæus* (West Africa).
Fig. 1.—Head of female, lateral view. Fig. 2.—End-on view of mouth. Fig. 3.—End-on view of teeth. Fig. 4.—Lateral view of mouth cavity. (Dorsal side on right side of plate). Fig. 5.—Ventral view of mouth cavity. Fig. 6.—Section of œsophagus. Fig. 7.—Tail of female. Fig. 8.—Tail of male, lateral view. Fig. 9.—Tail of male, ventral view.


1
6
4
5
·05 mm
7
·5 mm
2
·1 mm
3
8
·5 mm
9

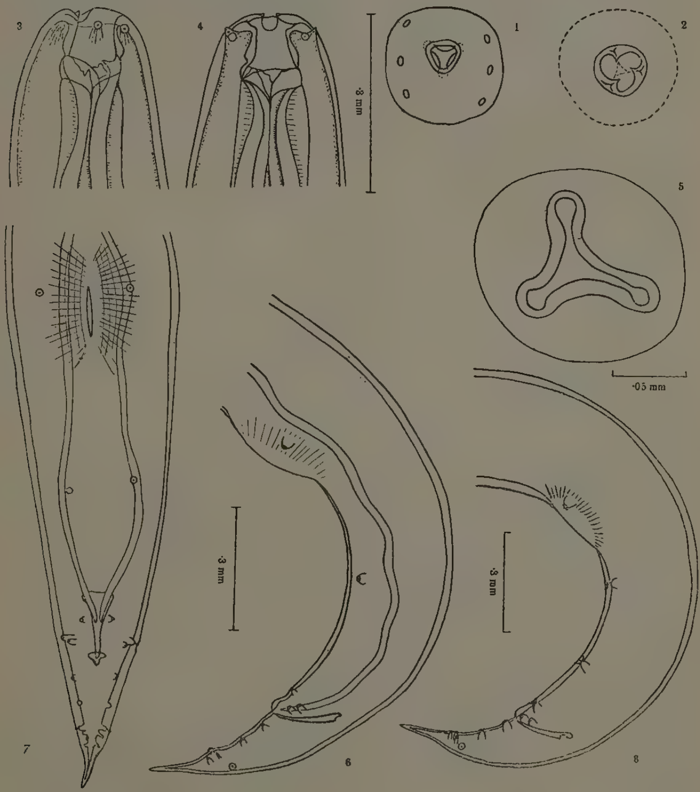
section (figs. 4 and 5). At the base of this cavity are three complicated teeth, arranged radially, with the axis of each tooth at right angles to the plates guarding the mouth (fig. 3) ; the channel between these teeth and the œsophagus is coloured black in the drawing. The mouth cavity communicates with a long narrow œsophagus about 3 to 3·25 mm. long in the female, and 2·5 mm. in the male which terminates in a posterior muscular bulb (fig. 1). The œsophagus in cross section, shows a triangular lumen lined by a dense, transversely striated cuticle. On the sides of the triangle are small diverticulæ (fig. 6), which run for the entire length of the œsophagus. The outline of the œsophagus is modified accordingly. The excretory pore is at the anterior quarter of the œsophagus ; while the nerve ring is at the level of the junction of the two portions.

The female. The vulva is a fairly conspicuous structure just anterior to the middle of the body. The vulva communicates with a long muscular ovijector, which terminates in a stout muscular valve. This portion is succeeded by a wider, less muscular portion which gradually gives place to the thin-walled uterus The common uterus quickly divides and the two branches diverge. The ovarian tubules fill the body space from just behind the œsophagus to posterior to the anus. The anus is situated about 1·6 mm. anterior to the tail which is sharply pointed and has no papillæ. The ova are large and thick shelled, and measure 43μ to 63μ long by 33μ to 30μ broad.

The male. The tail is always curved ventrally and sharply pointed. At about 1 mm. from the tip of the tail is the muscular, non-cuticular sucker, about 0·3 mm. long. On either side of the middle of the sucker is a large fleshy papilla. Midway beween it and the ano-genital opening, is a second pair (figs. 8 and 9). At the tip of the tail are three pairs of medium size papillæ and one pair of slender papillæ. Just posterior to the ano-genital opening are two pairs of large papillæ : and just anterior are three pairs of similar papillæ—two being lateral and one median in position. In all there are eleven pairs of papillæ in the male and this number appeared to be constant. There are two long slender spicules equal in size and similar in shape, about 2 mm. long. The free ends are generally bluntly pointed, but may bifurcate ; the anterior ends are blunt and thickened. Both spicules and spicular sheaths are transversely striated.

The accessory piece is short and straight, being pointed at the posterior end and swollen and excavated at the anterior end. It is 0·18 mm. long.

The testicular tube is laterally coiled in the middle of the body but is relatively straight anteriorly.



Subulura neodistans from *C. sabæus* (West Indies).

Fig. 1.—Head, end-on view of mouth. Fig. 2.—Head, end-on view of teeth. Fig. 3.—Lateral view of mouth cavity. (Dorsal side on left of plate). Fig. 4.—Dorsal view of mouth cavity. Fig. 5.—Section œsophagus. Fig. 6.—Lateral view of tail of male. (11 papillæ.) Fig. 7.—Ventral view of same. Fig. 8.—Lateral view of tail of male. (12 papillæ.)

SUBULURA NEODISTANS sp. nov.

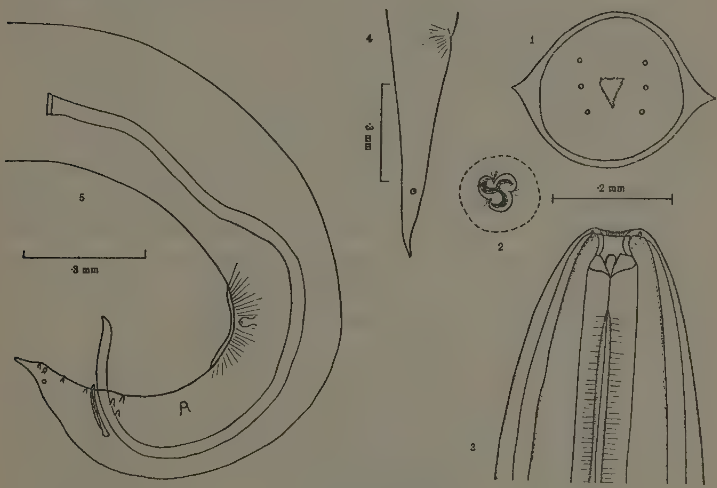
The island of St. Kitts in the West Indies is overrun with wild monkeys (*C. sabæus*) which were originally introduced from West Africa. There are records of these herds of monkeys being a public nuisance over two hundred and fifty years ago, and it appears safe to estimate that it is three hundred years since they left West Africa. Almost without exception, they harbour a species of *Subulura* in the cæcum, colon—and occasionally in the stomach. This form, while agreeing in its general configuration with *S. distans* of African origin, presents several small, but constant differences from that species. The most conspicuous is the shape of the lumen of the œsophagus. The various differences, referred to below, seem sufficient to justify the creation of a new species.

I have examined a very large number of specimens of *Subulura* from St. Kitts and without exception, all have a plain triangular lumen to the œsophagus (fig. 5) ; at the same time, I have had the opportunity of examining specimens from a variety of African hosts and in all cases, the longitudinal cylindrical diverticulæ were present. It is possible that in the St. Kitts' monkeys, there has been preserved an African species of *Subulura* which has since died out in Africa or which has not been represented among the specimens at my disposal. It is also possible, however, that the three hundred years isolation in a new environment has been sufficient time for the establishment of a new species. The variations in the disposition of the caudal papillæ in the male between eleven and twelve (no other member of this genus is known with more than eleven pairs of caudal papillæ), supports this latter hypothesis. The evidence, however, is still insufficient to make any definite pronouncement.

The females vary in length from 28 mm. to 32 mm. and in breadth from 0·46 to 0·6 mm., while the males are 15 mm. to 23 mm. long by 2·5 mm. wide. The body shape is similar to *S. distans*. Cervical alæ are absent. The mouth opening (fig. 1) is triangular with reinforcing plates on the sides. It is smaller in size than *S. distans*. The mouth cavity is about the same size, but the teeth are more regular in outline. The œsophagus is about 2·5 mm. long and is regularly circular in outline. The lumen is very heavily cuticularised (fig. 5) and forms a regular triangle with rounded angles. The morphology of the female is similar to *S. distans*. The tail is 1·2 mm. long in a

specimen 30 mm. long.

The male has the same general appearance as *S. distans*. The spicules are identical and measure 1·8 to 2·2 mm. long. The accessory piece is about 0·18 mm. long. In about a third of the specimens examined there are twelve pairs of papillæ present (fig. 8), the extra pair being in the pre-anal group. Occasionally there are eleven on one side and twelve on the other; in one specimen there were twelve on one side and thirteen on the other.



Subulura jacchi.

Fig. 1.—End-on view of mouth. Fig. 2.—End-on view of teeth. Fig. 3.—Lateral view of head. Fig. 4.—Tail of female. Fig. 5.—Tail of male.

Subulura jacchi.

This species has been previously reported from *Hapale jacchus*, *H. chrysoleucos*, *H. argentata* (= *melanura*), "*Midas*" *bicolor* and *Callicebus caligatus*. I had at my disposal material collected from *Hapale jacchus* as well as from *Hapale pygmæa* which had died at the London Zoological Gardens.

This species differs considerably from the previous forms. The female varies in length from 12 to 18 mm. and the male from 10 to 15 mm.

The width is 0·5 to 0·6 mm.

Lateral cervical alæ (figs. 1 and 3) are present in the œsophageal region. The mouth opening is small, roughly triangular but with no cuticular plates. There are six circumoral papillæ, all approximately the same size arranged in two rows. The base of the mouth cavity is occupied by the usual three radially arranged teeth, which are rounder and less irregular than in the other species. The œsophagus—about 1 mm. long—is similar to the other species. The lumen is triangular with sharply pointed angles. The excretory pore is in the second quarter of the œsophageal region and the nerve ring is at the junction of the straight part of the œsophagus and the bulb.

The female resembles the other species. The vulva is just anterior to the middle of the body. The tail is about 0·7 mm. long and sharply pointed (fig. 4) and carries two small caudal papillæ.

The male has, in general the same type of tail as in the other species. The sucker is rather larger in proportion. There are eleven pairs of papillæ arranged as in *S. distans*. The two pre-anal are very large and conspicuous: the post-anal and adanal are small and discrete. The spicules are equal and similar and about 1·7 mm. long. The accessory piece is gently curved and measures 0·2 mm. long.

This species is easily distinguished from the African forms by the presence of cervical alæ and of an unarmed mouth opening.

SUBULURA SARASINORUM (MEYER 1896) FROM LORIS GRACILIS.

The male is 7·5 mm. long, the female 10 to 11 mm. Conspicuous cervical alæ are present in the œsophageal region only. The œsophagus is 1·4 mm. long. The excretory pore is near the mouth and the nerve collar just in front of this. The mouth is almost circular and is stated to have four teeth and four papillæ. The œsophagus is swollen posteriorly with a valvular bulb, but no constriction is shewn in the drawing.

The male has caudal alæ and ten pairs of papillæ—four pre-, and six post-anal. The spicules are equal, 2·5 mm. long and 0·016 mm. wide.

The female has the vulva in the middle of the body. The eggs are $81\mu \times 65\mu$.

SUBULURA OTOLICNI (V. BENEDEN, 1890) FROM GALAGO SPP.

The male is 8 mm. long, the female 12·5 to 16 mm. long. The lips

are indistinct with the usual six circum-oral papillæ. There are three chitinous teeth in the buccal cavity The œsophagus is 1·2 to 1·3 mm. long with an œsophageal bulb (containing the usual valvular arrangement) but with no constriction between the two portions. The nerve ring surrounds the œsophagus about the junction between the first and second quarters.

The male has two small alæ. There is a sucker present about 0·48 mm. in front of the anus. There are eleven pairs of papillæ of which three are pre-anal. The spicules are 1·8 mm. long; the accessory piece is 0·225 mm. long.

In the female, the anus opens 1·4 mm. from the tail, which is sharply pointed. The vulva is in the middle of the body which it divides in the ratio of 7 : 9. The eggs measure 66μ by 50μ and are embryonated.

It is possible that the study of fresh material of this and the previous species, will necessitate their transference to a new genus. Meanwhile, however, they are retained in the genus *Subulura*, as the reported absence of a posterior bulb to the œsophagus is probably an error.

Subulura perarmata (Ratzel, 1868) from Tarsius tarsius.

This species is very incompletely known. The male is 6 to 7 mm. long, the female 8 to 10 mm. long. The width is 0·4 mm. There are stated to be six buccal teeth—three large and three small. The œsophagus is one-eighth of the body length and the bulb is 0·15 mm. in diameter.

The male has sharp pointed identical spicules 2·5 mm. long. The accessory piece is 0·15 mm. long and the sucker is 0·5 mm. from the tip of the tail. The number of papillæ is not stated, although v. Drasche mentions that there are six pre-anal and six or seven post-anal. The vulva in the female is stated to be just behind the middle of the body.

While it seems extremely probable that this species from *Tarsius* is correctly referable to the genus *Subulura*, the description is too scanty to allow of any comparison with the others. The name is retained, however, in the meantime.

Distribution.

The distribution of the genus *Subulura* is peculiar. *S. distans* and *S. neodistans* are confined to the old world genus, *Cercopithecus* and the very closely related genera *Erythocebus* and *Cercocebus*. *S. jacchi* is confined to marmosets and *Callicebus caligata* a fact that suggests that

this latter species, now placed in the Cebidæ, is very closely related to the Hapalidæ—physiologically if not morphologically. There are no other records of *Subulura* from monkeys, except the three forms briefly described from *Loris*, *Galago* and *Tarsius*. Among the Cercopitheques and marmosets, however, it is a very common parasite.

References.

Barreto, A. L., 1919.—" Sobre as especies brasileiros da sub-familia Subulurinæ Travassos, 1914." Mem. Inst. Oswaldo Cruz., Vol. xi., pp. 10-70. (*W.L.* 13465.)

Molin, R., 1860.—" Trenta specie di Nematoidi." *Sitz Ber. Akad. Wiss. Wien.*, Bd. 40. (*W.L.* 20170.)

Journal of Helminthology, Vol. VIII., *No.* 1, *February*, 1930, *pp.* 59-64.

On a collection of Cestodes from Nigeria.

By Ch. Joyeux and Jean G. Baer.
(*Laboratoire d'évolution des êtres organisés, Paris.*)

The following collection of Cestodes has been brought together by Prof. A. S. Pearse, of the Duke University, Durham, N.C., during a sojourn in Nigeria for the purpose of studying the parasites of rodents and insectivores.

We are indebted to Prof. Pearse for this carefully prepared collection containing several species new to science.

The hosts have been determined by Major C. M. Ingoldby of the British Museum.

The following is a list of the species determined :

Anoplocephalidæ Fuhrmann, 1907.

Inermicapsifer arvicanthidis (Kofend, 1917).

This species was found in the following hosts : *Hybomys univittatus* Pet., *Lemniscomys striatus* L., *Mastomys erythroleucas* Temm., *Taterona kempi* Wroughton. It appears to be very common in tropical Africa, but is also found, though scarcer, in South Africa.

Inermicapsifer guineensis (Graham, 1908).

Found in *Arvicanthis rufinus* Thomas, *Cricetomys buchanani* Thomas, *Cricetomys emini* Wroughton. This species is common in West Africa and has been reported by several authors.

Oochoristica herpestis Kofend, 1917.

Found in *Atelerix spiculus* Thomas and Wroughton, *Atelerix spinifex* Thomas. This species was found on a single occasion by Kofend (1917) in the Sudanese mongoose *Herpestes sanguineus* Rüpp. It has never been found since. Our specimens appear to be identical with those

described by Kofend, the hosts are, however, totally different. We do not believe it possible that a Cestode from a carnivore can also live in insectivora unless accidentally. This appears to be the explanation in the present case. It would seem that *O. herpestis* is a normal parasite of insectivora, where we have found it on two occasions, and is only found in carnivora when the latter have eaten insectivora. On the other hand if *O. herpestis* should be found on several occasions in carnivora it would seem necessary to create a new variety for it.

DAVAINEIDÆ Fuhrmann, 1907.

RAILLIETINA (RAILLIETINA) BAERI Meggitt and Subramanian, 1927.

Found in *Funisciurus anerythrus* Thomas, *Malacomys edwardesi* Rochebr., *Mastomys erythroleucas* Temm., *Mastomys sp.*, *Praomys tullbergi* Thomas. This species has already been found in Rats from the Gold Coast and from Dahomey. The type of this species has 60-65 hooks. We have been able to observe a considerable variation as the following figures show: 30, 40, 46, 60.

RAILLIETINA (RAILLIETINA) TRAPEZOIDES (Janicki, 1904).

Found in *Arvicanthis mordax* Temm., *Lemniscomys striatus* L., *Taterona kempi* Wroughton. This species appears to have a very wide distribution. It has been reported from Egypt (Janicki, 1904), from the Kalahari (Hungerbühler, 1910) and by ourselves from southern Algeria (1929).

HYMENOLEPIDIDÆ Railliet and Henry, 1909.

HYMENOLEPIS SCALARIS (Dujardin, 1845).

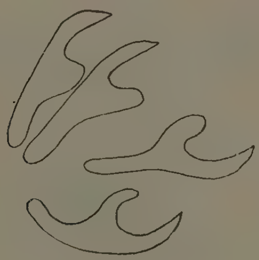
Found in *Crocidura sp.* Seems to have a very wide geographical range.

HYMENOLEPIS PEARSEI n. sp.

Found in *Hybomys univittatus* Pet. This new species measures about 100 mm. in length with a maximum width of 2 mm. The scolex is 0·29 mm. in diameter, each of the four suckers measures 0·1 mm. The rostellum is armed with a single crown of ten hooks. The shape of the hooks is seen in the figure, their measurements are as follows: Length 69μ from the tip of the handle to the tip of the point. Base 56μ from the tip of the guard to the tip of the handle. We prefer to figure several hooks drawn from different angles, instead of giving complicated and unreliable formulæ. The anatomy presents the typical characters of

the genus *Hymenolepis*, as can be seen from our figure. The female genitalia are situated in the poral half of the segment. There is a very large receptaculum seminis. The cirrus pouch is 0·42 mm. in length and 0·06 mm. in diameter. The cirrus is armed with small spines. The ova measure 65μ in diameter and the embryos 30μ.

This species can easily be differentiated from the other species of *Hymenolepis* inhabiting rodents, by the size and shape of the rostellar hooks. We dedicate this new species to Prof. A. S. Pearse.



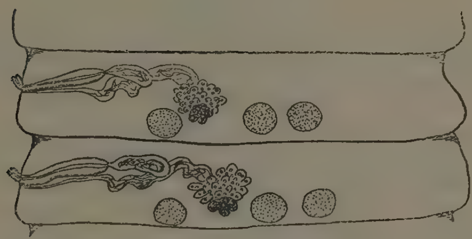


Fig. 1.—*Hymenolepis pearsei*. Several rostellar hooks.
Fig. 2.—*Hymenolepis pearsei*. Two segments showing the internal anatomy.

HYMENOLEPIS UNCINISPINOSA n. sp.

Found in *Hybomys univittatus* Pet., *Mastomys erythroleucas* Temm. The length appears to be about 50-80 mm. and the greatest width 1 mm. The scolex is armed with a single row of twelve hooks of a typical rose-thorn shape. These hooks measure 34μ from the tip of the handle to

the tip of the point and 37μ from the tip of the handle to the tip of the guard. The anatomy resembles somewhat that of *H. pearsei*, the three testes are however situated close together, behind the female genitalia. The cirrus pouch measures 0·15 mm. in length and 0·02 mm. in diameter. The ova measure 53μ by 38μ and the embryos 38μ by 23μ.

This species appears to be closely related to *H. globirostris* Baer, 1925, the number and the shape of the hooks is the same, type 4 (FUHRMANN). In the following table we give the characters separating the two species :—

	H. uncinispina n.sp.	*H. globirostris* Baer, 1925.
Length of hooks ...	34μ	24μ
Base of hooks ...	37μ	18–18·4μ
Length of cirrus pouch ...	0·15 mm.	0·26 mm.
Ova	53μ by 38μ	30μ
Embryo	38μ by 23μ	20μ
Distribution ...	Nigeria	Belgian Congo

TAENIIDÆ Ludwig, 1886.

CATENOTÆNIA LOBATA Baer, 1925.

Found in *Mastomys erythroleucas* Temm., *Taterona kempi* Wroughton. This species seems to have a very wide geographical distribution. It has been found several times in tropical Africa and has been reported from England by BAYLIS (1926).

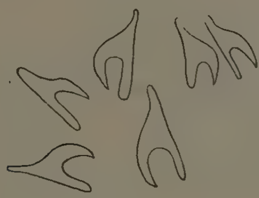
TAENIA TAENIÆFORMIS Batsch, 1786.

The larval stage of this tapeworm was found in the liver of a rat, *Rattus rattus* L. It has already been reported from tropical Africa by ourselves (1928).

Diphyllobothriidæ Lühe, 1910.

Duthiersia fimbriata (Diesing, 1854).

Found in *Varanus sp.* This species is extremely frequent in all the African monitors.



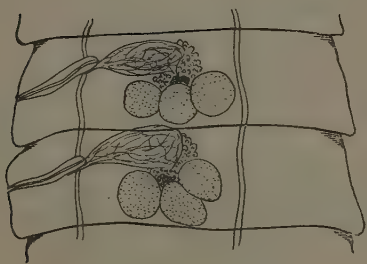


Fig. 3.—*Hymenolepis uncinispina.* Rostellar hooks.
Fig. 4.—*Hymenolepis uncinispina.* Internal anatomy.

Conclusions.

The above collection of tapeworms from Nigeria is of very great interest. Thirteen of the hosts mentioned have never been reported as harbouring cestodes. Three of the hosts, *Atelerix spiculus* Thomas and Wroughton, *Atelerix spinifex* Thomas, and *Crocidura sp.*, are insectivora, all the other hosts are rodents. With regard to the latter we wish to draw attention to the fact that south of the Sahara *Hymenolepis fraterna* Stiles, 1906, the common cestode of rats in temperate or in Mediterranean climates, is very scarce and seems to be replaced by species of the genera

Inermicapsifer and *Raillietina*. On the other hand *Hymenolepis nana* (v. Siebold, 1852) is found in man in all tropical climates.

References.

Baer, J. G., 1925.—" Sur quelques cestodes du Congo belge." *Rev. suisse Zool.* xxxii., pp. 239-251. (*W.L.* 19288.)

— 1927.—" Monographie des cestodes de la famille des Anoplocephalidæ." *Bull. biol.*, Supp. x., pp. 241. (*W.L.* 3919.)

Joyeux, Ch., Gendre, E., Baer, J. G., 1928.—" Recherches sur les Helminthes de L'Afrique occidentale française." *Collection de la Soc. Path. exot. Monographie*, ii., 120 pp.

Kofend, L., 1917.—Cestoden aus Saügetiere und aus *Agama colonorum*." *Anz. Wien. Akad. math.-naturwiss.*, lxiv., pp. 229-231.

Meggitt, F. J., and Subramanian, K., 1927.—" The tapeworms of Rodents of the subfamily Murinæ, with special reference to those occurring in Rangoon." *J. Burma Res. Soc.*, xvii., pp. 190-237. (*W.I.* 11091.)

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp.* 65-68.

J. G. DE MAN (1850-1930).

By the death of Dr. J. G. de Man which took place at Middelburg, Holland, on January 19th, 1930, zoology has lost a specialist of world-wide reputation in two great groups of the animal kingdom. His published works were devoted, almost entirely, to studies on free-living nematodes and the decapod crustacea.

Johannes Govertus de Man was born at Middelburg, Holland, on May 2nd, 1850, so that at the time of his death he was within a few months of his 80th birthday. He took his Ph.D. degree at the University of Leiden in 1873. The summer months of 1872 were spent at Leipzig in Leuckart's laboratory. In the same year he became first assistant in the National Museum of Natural History at Leiden and in 1875 was promoted to conservator of the museum. Whilst working at Naples in 1876 he became seriously ill with typhoid fever and a further illness in 1883 necessitated his resignation from his position at the museum. He retired to private life and continued his researches for 10 years at Middelburg and from 1893 onwards at Jerseke.

Early in the present century he undertook the study of the decapod crustacea collected by the " Siboga " expedition and this work occupied much of his time for about twenty years. His numerous Helminthological publications were issued over a period of more than 50 years from 1876 onwards. Some are short papers but others are extensive monographs liberally illustrated with excellent drawings. In every case the work is of a high order ; the careful descriptions of numerous genera and species of soil, fresh-water and marine forms, revealing accurate and painstaking investigation. His drawings also were always beautifully finished and show anatomical features in considerable detail. His great work " *Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden,*" published in 1884 and re-issued in 1919, will always remain a classic and a standard work of reference.

To judge from his letters de Man must have been of a most amiable disposition, self-effacing and genuinely devoted to a life of scientific investigation. They were excellently written in English and were always most courteous and kindly. He was ever willing to help and to discuss in a friendly manner details about " my beloved nematodes," as he called them in one letter.

On one occasion his supply of reprints of a particular paper, having run out, he copied the relevant portions and sent them to his correspondent along with tracings of his drawings ; such a generous act one felt to be characteristic of the man.

He was decorated as Officer of the Order of Cambodia by the President of the French Republic in April, 1901, and by the Queen of Holland with the Star of the Orange, Officer of the Order of Nassau in September, 1923.

The accompanying photograph shows him at about the age of 50 and is a good likeness. The writer is greatly indebted to Miss M. G. A. de Man, sister of the late savant, for most of the foregoing particulars of Dr. de Man's career.—T.G.

HELMINTHOLOGICAL WORKS.

1876.—" Onderzoekingen over vrij in de aarde levende Nematoden," *Tijdschr. ned. dierk. Ver., Deel* 2, *pp.* 78-196.

1876.—" Contribution à la connaissance des nématoïdes marins du Golfe de Naples," *Ibid, Deel,* 3, *pp.* 88-118.

1880.—" Die einheimischen, frei in der reinen Erde und im süssen Wasser lebenden Nematoden (der Niederländischen Fauna), monographisch bearbeitet. Vorläufiger Bericht und descriptiv-systematischer Theil," *Ibid., Deel* 5, *pp.* 1-104.

1881.—" Ueber einige neue oder noch unvollständig bekannte Arten von frei in der reinen Erde lebenden Nematoden," *Ibid, Deel* 5, *pp.* 138-143.

1884.—" Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der Niederländischen Fauna. Eine systematisch-faunistische Monographie." Leiden.

1885.—" Helminthologische Beiträge," *Tijdschr. ned. dierk. Ver.*, (2) *T.I. pp.*. 1-26

1886.—" Anatomische Untersuchungen über freilebende Nordsee-Nematoden." Leipzig.

1886.—" Rapport sur le mémoire du Docteur Lad. Oerley : Die Rhabditiden und ihre medizinische Bedeutung," *Biol. Zbl., pp.* 433-435.

1888.—" Sur quelques Nématodes libres de la Mer du Nord nouveaux ou peu connus," *Mém. Soc. zool. Fr., T.I. pp.* 1-51.

1888.—" Diseased Growth in Oat Plants," *Agricultural Gazette, p.* 61, London.

1889.—" Ueber zwei in der feuchten Erde lebende Arten der Gattung *Oncholaimus* Duj.," *Tijdschr. ned. dierk. Ver.,* (2) *T.* 11.

1889.—" Espèces et genres nouveaux de Nématodes libres de la Mer du Nord et de la Manche," *Mém. Soc. zool. Fr., T.* 2, *pp.* 1-10.

1889.—"Troisième note sur les Nématodes libres de la Mer du Nord et de la Manche," *Ibid., pp.* 182-216.

1890.—" Quatrième note sur les Nématodes libres de la Mer du Nord et de la Manche," *Ibid., T. 3. pp. 169-194.*

1892.—" Ueber eine neue in Gallen einer Meeresalge lebende Art de Gattung *Tylenchus* Bast.," *Festschrift zum siebenzigsten Geburtstage Rudolf Leuckarts, pp.*121-125.

1893.—" Cinquième note sur les Nématodes libres de la Mer du Nord et de la Manche," *Mém. Soc. zool. Fr. T. 6, pp. 81-125.*

1894.—" Note supplémentaire dur la *Rhabditis janeti* Lac. Duth," *Ibid., T.* 7 *pp.* 363-371.

1895.—" Description of three species of Anguillulidae, observed in diseased pseudo-bulbs of tropical Orchids," *Trans. Lpool Biol. Soc., pp.* 76-94.

1903.—" *Pseudomermis Zikoffi*, nov. gen., nov. spec.," Zykoff W. Material po fauny, Volghi i ghidrofaunye Saratovskoi. *Bull. Nat. Moscou, No.* 1, *pp.* 61-64.

1904.—" Nématodes libres," *Expédition Antarctique Belge. Resultats du Voyage du S. Y. Belgica en 1897-1898-1899. Zoologie, Anvers,* 51 pages.

1904.—" Ein neuer freilebender Rundwurm aus Patagonien. *Plectus* (*Plectoïdes*) *patagonicus,* n.sp.," *Ber. sen Kenb. naturf. Ges., pp.* 41-45.

1906.—" Sur quelques espèces nouvelles ou peu connues de Nématodes libres vivant sur les côtes de la Zélande," *Tijdschr. ned. dierk. Ver.* (2) *Dl,* 10, *pp.* 227-244.

1906.—" Observations sur quelques espèces de Nematodes terrestres libres de l'île de Walcheren," *Ann. Soc. zool. malac. Belg.* T. 41, pp. 156-174.

1907.—" Contribution à la Connaissance des Nématodes libres de la Seine et des environs de Paris," *Ann. Biol. lacust, T.* 2, *pp.* 9-29.

1907.—" Sur quelques espèces nouvelles ou peu conneus de Nématodes libres habitant les côtes de la Zélande," *Mém. Soc. zool. Fr., T.* 20, *pp.* 33-90.

1908.—" *Plectus ?* sp., F. Richters, Beitrag zur Kenntnis der Moosfauna Australiens und der Inseln des Pazifischen Ozeans," *Zool. Jahrb. Abt. Systm. Bd.* 26, *pp.* 199-202.

1908.—" Note on a free-living Nematode from Rham-Tso Lake, Tibet," *Rec. Ind. Mus., Vol.* 2, *Pt.* 4, *pp.* 314-315.

1910.—" Beiträge zur Kenntnis der in dem weissen Schleimfluss der Eichen lebenden Anguilluliden, nebst Untersuchugen über den Bau des Essigälchens und der Gattung *Anguillula* Ehrb," *Zool. Jahrb. Abt. Systm. Bd.* 29, *pp.* 359-394.

1912.—" Helminthologische Beiträge," *Zool. Jahrb. Suppl.* 15, *Bd. 1, pp.* 439-464.

1912.—" *Odontopharynx longicaudata* n.g., n.sp., Eine neue Form von Anguilluliden," *Zool. Jahrb. Abt. Systm.* Bd. 33, pp. 637-642.

1913.—" *Anguillula silusiae* n.sp., eine neue, in den sogenannten ' Bierfilzen ' lebende Art der Gattung *Anguillula* Ehrb," *Zbl. Bakt. Abt.* 2, *Bd.* 39, *pp.* 73-74.

1914.—" *Anguillula silusiae* de Man. Eine neue in den sogenannten ' Bierfilzen ' lebende Art der Gattung *Anguillula* Ehrb," *Ann. Soc. zool. malac. Belg. T.* XLVIII, *pp.* 1-9.

1917.—" Beitrag zur Kenntnis der in Norwegen frei in der reinen Erde lebenden Nematoden." *Tijdschr. ned. dierk. Ver.* (2) *Dl.* 16, *p.* 103-119.

1919.—" Die, frei in der reinen Erde und in süssen Wasser lebenden Nematoden der Niederländischen Fauna. Eine systematischfaunistische Monographie."

1920.—" Description d'une espèce nouvelle du genre *Diplogaster* Max Schultze : *Diplogaster consobrinus.* nov. sp,." *Ann. Soc. zool. malac. Belg. T.* LI, *pp.* 47-54.

1921.—" Nouvelles recherches sur les Nématodes libres terricoles de la Hollande," *Capita zool. Dl, 1, Afl. 1.*

1922.—" Lijst van gedurende de laatste vijf jaren in de provinciën Zuid-Holland, Noord-Brabant en Zeeland waargenonem terricole Nematoden," *Tijdschr. ned. dierk. Ver.* (2) *Dl.* 18.

1922.—" Neue frei lebende Nematoden aus der Zuidersee," *Ibid., pp.* 124-134.

1922.—" Über einige marine Nematoden von der Küste von Walcheren, neu für die Wissenschaft und für unsere Fauna, unter welchen der sehr merkwürdige *Catalaimus Max Weberi* n.sp.," *Bijdr. Dierk, Afl. 22, pp.* 117-124.

1923.—" Vrij levende Nematoden," *Flora en Fauna der Zuiderzee. Monografie van een Brakwatergebied, uitgegeven door de Nederlandsche Dierkundige Vereeniging ter gelegenheid van haar vijftigjarig bestaan.*

1926.—" Beschreibung einer neuen Art der Gattung *Craspedonema* Richters, *Craspedonema zeelandicum,* n.sp.," *Arch. Hydrobiol. Plankt. Bd.* 17, *No.* 3, *pp.* 507-514.

1927.—" Das Männchen der *Rhabditis monhystera* Bütschli," *Zool. Anz.* 70, *pp.* 51-57.

1928.—" Sur quatres espèces de Nématodes libres observées aux environs de Caen dans un canal d'eau saumâtre à Ouistreham," *Bull. Soc. zool. Fr. T.* LIII, *pp.* 96-103.

1929.—" On a New Cavernicolous Species of the genus *Dorylaimus* Duj.," *F. M. S. Mus. J. Vol.* XIV, *Pts.* 3 & 4, *pp.* 352-355.

Journal of Helminthology, Vol. viii., No. 2, June, 1930, pp. 69-76.

The Helminth Parasites of the Goat in Britain, including an account of *Skrjabinema ovis* (Skrjabin, 1915), Werestchagin, 1926.

By D. O. MORGAN, M.Sc., Ph.D.

(*Senior Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

INTRODUCTION.

OWING to the comparatively little use made of the flesh of the goat in this country, the helminthic parasites of this host have been far less studied than those found in other domestic animals. In other countries, where the goat plays a far greater economic rôle, a large number of parasitic worms have been recorded.

While it is the case that worms which cause death or serious loss of condition, such as lung and stomach worms, are quite well known in goats in Britain, there are few records of the numerous other species which occur in this host. These latter species, although not the direct cause of serious depletion in a herd, may, however, cause unthriftiness, loss of milk and retarded growth.

So far, fifty-three different species of worms have been recorded from the goat in various countries. Many of these are not found in this country and it is unlikely that there are any species entirely confined to the British goat. It is true that the goat in this country is somewhat isolated owing to the restrictions which exist on importation. A few goats have, however, been imported at long intervals, and it seems possible that at least one of the species mentioned in this paper, viz. *Skrjabinema ovis*, has been introduced from abroad in this way.

Although the species may be the same, the frequency and intensity with which certain worms occur may vary considerably between one

country and another. This is due to differences in climate and to differences in the methods of goat husbandry. The problems which, therefore, are of the greatest importance in one country may be of much less consequence in another.

A knowledge of the distribution of goat parasites in this country is also of value since this host can spread infection to other domestic animals and *vice versa*. In fact the majority of the worms recorded from the goat are also common parasites of sheep and, to a less degree, of cattle also.

Of the importance of worms in relation to the health of the goat in Britain there can be no doubt, as a study of the more recent literature on this question will shew. Stainton (1928) states :—" From the number of cases of parasitic gastritis and infestation by worms in general which the writer has encountered recently, it would appear that the question of parasitism in the goat certainly needs some attention." The danger from worms is undoubtedly greatest where large herds of goats are kept, and particularly where over-stocking occurs. The isolated goat would appear to be in the safest position with regard to worm infestation. Since, however, the majority of goats are not entirely free from worms, the cottager's goat, if tethered too frequently on the same spot, would soon cause a small area to become heavily contaminated. Such an infective centre would be dangerous not only to the goat itself but particularly so to its young kids.

Parasites Found in British Goats.

During the last two years the Institute of Agricultural Parasitology has been able, through the co-operation of the members of the British Goat Society, to make an examination of some 36 goats obtained from various parts of the country and it is thought that a record of the species of helminths found in the animals hitherto examined might be of some interest.

Trematodes.

No Trematodes were obtained by the writer in any of the goats examined. This is probably due to the fact that the goats were not obtained from the localities where flukes are commonly found in other domestic animals,

namely the West and North. *Fasciola hepatica* has, however, been recorded from goats in Scotland by Cameron (1923).

CESTODES.

A few specimens of the bladder worm *Cysticercus tenuicollis* were found in three different goats.

Among the adult members of this group, twelve young adults of *Moniezia expansa* were found in one goat. This is the only record of adult Cestodes apart from some segments of tapeworms found in the droppings of goats on the field plots.

NEMATODES.

By far the greatest number of species found came under this group. These are arranged below according to their habitat in the host.

Parasites of the Lung.—As in the case of sheep in this country, *Muellerius capillaris* was a common parasite of the lung and it frequently occurred in large numbers. On the other hand *Dictyocaulus filaria* was only found on one occasion in the goats examined when it was obtained from a young kid.

Parasites of the Abomasum. The prevalence of parasitic gastritis in British goats has already been referred to and it was not surprising, therefore, that *Hæmonchus contortus* and *Ostertagia circumcincta* were more commonly met with than any other Nematode. The other parasites of the abomasum viz. *Ostertagia trifurcata* and *Trichostrongylus extenuatus* were much more rare.

Parasites of the Small Intestine.—Among the worms found in this region of the alimentary canal, *Trichostrongylus colubriformis*, *T. vitrinus* and *T. capricola* were very common, while *Capillaria longipes*, *Cooperia curticei* and *Nematodirus filicollis* were not so frequently met with. The writer did not find the hookworm *Bunostomum trigonocephalum*, although this parasite is very common in sheep in this country. This species has, however, been recorded by Cameron (1923) in goats from the Pentland Hills.

Parasites of the Caecum and *Large Intestine.*—In this region were found *Œsophagostomum venulosum*, *Chabertia ovina* and *Trichuris ovis*. These parasites were of frequent occurrence and particularly so in the case of *Œ. venulosum*. This latter species has been wrongly referred to as the

cause of " pimply gut " in sheep and goats, whereas the species associated with this condition is *Œ. columbianum*. This worm has not been found, as far as the writer is aware, either in sheep or goats in this country.

Another species found in the large intestine of two of the goats examined was *Skrjabinema ovis*. The occurrence of this parasite in England is remarkable since, apparently, the only other records are from the sheep, goat and gazelle in Turkestan. The publications in which this species was originally described are difficult to obtain in this country and it was thought, therefore, that a new description of the parasite based on the specimens found in British goats might be of some use.

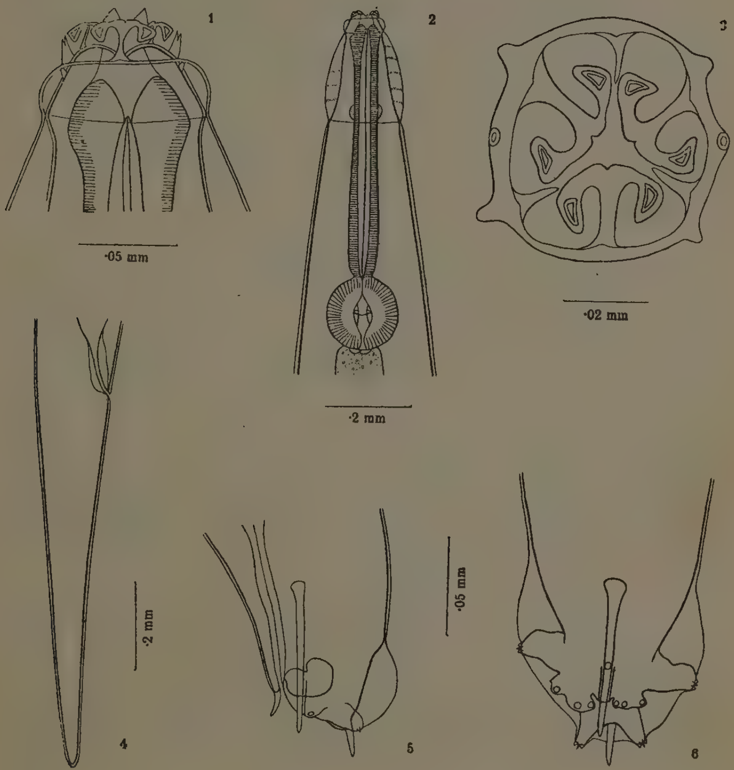
Skrjabinema ovis.

Skrjabinema ovis was first found by Skrjabin in sheep from Turkestan in 1915. Only the female was found by Skrjabin and on this account he was only able to place his species in the genus Oxyuris (sensu lato) under the name of *Oxyuris ovis*. In 1926, Werestchagin, who found both male and female of the species in a collection of goat parasites from Turkestan, erected the new genus *Skrjabinema* to take this parasite. Later, Schulz (1928) found the species in another host, viz. *Gazella subgutturosa* from the same locality.

The worms were obtained by the writer from the large intestine of two kid goats which had been sent to this Institute from the South of England. The goats had been kept on the observational plots for some months before being examined and it is, therefore, difficult to say whether infection had occurred previous to their arrival or not. The fact that fairly young stages of the parasites were found seemed to shew that, at any rate, reinfection was taking place on the Institute plots.

Owing to the restrictions on the importation of goats it is somewhat remarkable that this parasite should occur in this country. Even in the few instances where goats have been allowed to be introduced they have been obtained from European countries where *S. ovis* does not appear to be present. On the other hand it may be that the parasite is of long standing in this country but has not been found before owing to the comparatively small attention given to the parasites of the goat. If this were so, however, one would expect to find the worm in sheep, but no record exists of its occurrence in this host in this country although a close study has been made of its parasites in several localities. On

the whole, therefore, its introduction from abroad seems the more probable supposition.



Skrjabinema ovis.

Fig. 1.—Head end of female in dorsal view.
Fig. 2.—Anterior end of female shewing œsophagus and spherical bulb.
Fig. 3.—End-on view of mouth region under high magnification.
Fig. 4.—Shewing female tail.
Fig. 5.—Tail end of male in lateral view.
Fig. 6.—Tail end of male in ventral view.

Morphology.

The female of *S. ovis* is straight and broad with a fairly long and slightly tapering tail. The male is much smaller, being only about half the length of the female, and has its posterior region distinctly curved ventrally. The cuticle at the head end shews a shallow inflation upon which the lips rest and is followed by a much broader and longer expansion. This expansion extends posteriorly to a point slightly behind the level of the nerve ring. There is also present a pair of narrow lateral alae which are transversely striated; the striations, however, are confined to these alae and do not encircle the body of the worm. The alae extend posteriorly for the greater part of the body length.

The mouth region is a complicated structure and the arrangement and character of the lips and papillæ are difficult to resolve. There are three lips present each of which shews a T shaped thickening of the cuticle with the two arms broadening out into a triangular expansion. At the summit of these lips are found two small processes which are directed downwards into the buccal cavity and are not easily visible when the lips are bent inwards towards the mouth opening. There are three interlabia present which appear to be cleft when viewed laterally. Three pairs of papillæ surround the mouth, one pair sub-dorsal, one lateral and the other sub-ventral. The laterals are short while the other two pairs are broader and longer and are only clearly visible in an end-on view of the mouth region.

The buccal cavity leads into a cylindrical œsophagus which terminates in a large spherical bulb with a valvular apparatus. The œsophagus measures 0·60 mm. in the female and the diameter of the bulb is 0·15 mm. In the male the œsophagus is 0·28 mm. and the bulb 0·1 mm. in diameter.

Female.—The female measures 6·37 mm. to 7·16 mm. in length with a maximum breadth of 0·31 mm. to 0·35 mm. At the head end the width is 0·08 mm. and the body reaches its maximum width in the region of the vulva. From this region there is a gradual diminution towards the tail, 0·16 mm. being the width at the anus. The tail is fairly long and measures 0·88 mm. It narrows gradually and evenly from the anus and ends in a blunt conical tip. The lateral alae end at a point between the anus and the tip of the tail about 0·5 mm. from the latter.

The vulva is situated about 2·34 mm. behind the head end and is marked by a short transverse slit. It leads into a broad vagina which is directed obliquely forward and measures 0·22 mm. in length. The excretory pore is situated between the bulb and the vulva about 1·49 mm. from the anterior end.

The eggs are typical of this group of nematodes, having one side convex and the other almost straight. They measure 0·058 mm. to 0·063 mm. in length and 0·030 mm. to 0·034 mm. in width.

Male.—The males have a length of 2·94 mm. to 3·07 mm. with a maximum width of 0·17 mm. The cervical expansion of the cuticle is much narrower in the male than it is in the female and the lateral alae are not so prominent. They extend, however, as in the female, to within a short distance of the posterior end of the worm and shew fine transverse striations.

The tail is bluntly rounded and has an almost circular caudal expansion of the cuticle which is supported by two pairs of processes. The larger pair of processes is preanal and lateral in position and bears three small papillæ, placed closely together, at the tip. The other pair is postanal and dorsal with a similar arrangement of papillæ at the summit. There is a further pair of processes present which are broad and shallow and situated adanally. These also bear three papillæ but the latter are placed further apart than those on the other processes.

In the mid-dorsal line the tail terminates in a short spike and there is a similar spike terminating the tail on the mid-ventral line.

A single, fairly stout spicule is present measuring 0·075 mm. to 0·095 mm. It has a club shaped head and from this point narrows evenly towards the pointed tip. There is also an accessory piece measuring 0·025 mm.

Remarks.

A study of the species recorded in this paper from British goats shews that, with two exceptions, viz. *S. ovis* and *T. capricola*, the worms found are also common parasites of sheep in this country.

The number of goats examined is too small to enable one to form any opinions as to the relative frequency of the different species in this country. In the animals examined *H. contortus*, *O. circumcincta* and

Œ. venulosum were the most common and were found in 14 individuals, while *C. ovina* and *T. ovis* occurred in 9 and *M. capillaris* in 8 of the goats.

These animals had been reared at Winches Farm on the observational plots attached to the Institute of Agricultural Parasitology and the incidence of these naturally acquired infections may be higher than among goats kept by owners and breeders in other parts of the country.

References.

Cameron, T. W. M., 1923.—" On the Intestinal Parasites of Sheep and other Ruminants in Scotland," *Jl. Helm.*, vol. i., No. 2, pp. 53-60.

Schulz, R. E., 1928.—" Sur la faune helminthologique de *Gazella subgutturosa* Gueld," *Ann. Parasit.*, T. vi., No. 1, pp. 101-104.

Skrjabin, K. I., 1915.—" *Oxyuris ovis* n. sp. un parasite nouveau du mouton," *Arch. de Sc. Vétér.*, vol. viii (in Russian).

Stainton, F. H., 1928.—" Parasites of the Goat," *Mon. Circ. Brit. Goat Soc.*, vol. xi., No. 10, pp. 5-7.

Werestchagin, M. N., 1926.—" Sur la faune helminthologique des chèvres du Turkestan," *Œuvres de l'Inst. de Méd. vét.*, vol. ii., L. 2 (in Russian).

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp.* 77-84.

Helminth Parasites of Stock in the British West Indies

By Thomas W. M. Cameron, M.A., D.Sc., M.R.C.V.S.

(*Lecturer in Helminthology in the University of Edinburgh.*)
(*From Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

During 1928 I was able, as Milner Research Fellow in the Department of Helminthology of the London School of Hygiene and Tropical Medicine, to make an extended survey of the parasites of man and the domestic animals in the British West Indies. A report on the former has already been published (1929). In examining the parasites of domestic animals, I was given every facility by the local authorities, and I am especially indebted to Captain H. V. Metivier, B.Sc., M.R.C.V.S., of Trinidad, whose assistance and knowledge of the local parasitic problems was invaluable.

The islands which were visited extended from Trinidad in the south to St. Kitts in the north and included the four Crown Colonies of Trinidad and Tobago, Barbados, the Windward and the Leeward Islands. They fall into three natural geological groups—sedimentary islands such as Trinidad, coral islands such as Barbados and part of Antigua, and volcanic islands, a group of which includes the great majority of the others. The configuration and type of soil varies extremely and practically no two are alike. In Trinidad and in parts of Barbados and of Antigua, clay plains are common and parasitism reaches a higher degree there than elsewhere. In the coral regions drainage as a rule is good and the soil is unsuitable for the development of helminths ; this is true also in the case of volcanic islands, although for different reasons. Where there is a coral substratum, water percolates rapidly through the soil ; where there is a volcanic soil, slopes are steep and water rapidly runs off the surface. In both classes accordingly, in spite of the abundant rainfall

and the tropical climate, conditions are not too favourable for the development of the parasitic worms and consequently individual infections are generally light. There are exceptions to this, of course, but these are isolated and relatively few and do not affect the general rule. Nevertheless, while the degree of parasitism is as a rule light, the percentage of animals harbouring helminths is high. There is however, no sensible variation in the parasitic fauna throughout the various islands, and they will accordingly be treated as a whole in this article, such minor differences as arise being noted under the various parasites.

The domestic animals are all imported and no native stock exists—in fact native animals of any description are rare. Three type of bovine animals are found—Indian buffaloes of various breeds, pure bred Zebu cattle, and crosses between Zebus and practically every known European breed of cattle. In recent years, especially in Trinidad, pure bred cattle have been introduced for breeding purposes (mainly from Canada), but these are few in number. I had no opportunity of examining any of the pure bred stock or buffaloes. Sheep are rare and are kept entirely for food purposes ; goats are more common and are used to supply both milk and meat. Pigs are found in all the island and are generally of a nondescript, " razor-back " variety. Horses, mules, and donkeys are also common. Every variety of horse from blood stock downwards is encountered ; but they are now gradually being displaced by mechanical transport.

No new species of helminths was found and the lists at the end of the article shew the species under the various hosts. Certain points of interest in connection with the distribution of the various forms are included in the following notes.

Cattle.

The parasites of oxen are relatively few, the commonest being *Setaria labiato-papillosa*, which, however, I only saw in Trinidad, although there is little doubt that it occurs elsewhere also. Amphistomes have been seen in the abattoirs on various occasions, but I was unable to secure specimens for examination. *Cysticercus bovis* occasionally is found as also is *Monodontus phlebotomus*, *Œsophagostomum radiatum* and

Dictyocaulus viviparus. I was unable to find any specimens of *Syngamus laryngeus* in any of the ruminants, although it has been reported from the Greater Antilles. Since a species of Syngamus (*S. kingi*) was reported by Leiper from man in St. Lucia, repeated search has been made in the Lesser Antilles by many workers for related forms in mammals, but without success.

Sheep and Goats.

The smaller ruminants are much more constantly parasitised than are the larger. Even here, however, the degree of parasitism was usually slight and as a rule only a few examples of any one species were recovered from any one animal. The most abundant helminth in the small intestine is *Trichostrongylus instabilis*; it comprised over 95 per cent. of all the species of Trichostrongyles collected. Most of the usual European species were occasionally encountered, but a surprising and notable exception was *Nematodirus*. Why this very common form should be absent is unexplainable. *Trichostrongylus instabilis*, in addition to being common in ruminants, also occurs in human beings and West African Green monkeys in St. Kitts.

The second most abundant helminth is *Muellerius capillaris* in the lung, a high percentage of sheep and goats seen in the abattoirs being infected.

Pigs.

No trematode parasites were found or have been recorded from the British West Indies; but *Cysticercus cellulosæ* is sometimes seen in the southern islands. On one occasion, I recovered a portion of a Moniezia from the small intestine of a pig in Trinidad; but it was obvious from its partly decomposed state in an intestine which was still warm, that it was not parasitic.

The most important of the nematodes occurring in West Indian pigs is undoubtedly *Stephanurus dentatus*. It generally occurs in the perirenal fat, but is not infrequently found in the liver also. It causes the condemnation of a large proportion of kidneys.

No less than four species of bursate nematodes occur in the alimentary canal; *Hyostrongylus rubidus* is found in the stomach, *Necator suillus* and *Globocephalus urosubulatus* in the small intestine and *Œsophagostomum dentatum* in the large intestine. Of the two species found in the small intestine, Necator is slightly more common in the southern islands than is Globocephalus; in the northern islands the ratio is reversed. Both species, however, are encountered in small numbers in all the islands, but no heavy infections were ever seen. Œsophagostomum is, however, sometimes found in very large numbers.

There has been a considerable amount of interest taken in the relationship between *N. suillus* and *N. americanus* and the public health significance of the former. Although I am unable to add anything to the description of the parasite given by Ackert and Payne (1923), the consistently smaller size of the porcine form suggests that it is at least, a biological variety distinct from those in man. This survey shews that it is not confined to Trinidad among these islands, but extends at least as far north as St. Kitts. I have also found it in pigs from Venezuela, and it is probable that its distribution will be found to be fairly extensive in neotropical regions. At the same time it is important to remember that at least four species of bursate nematodes inhabit the alimentary tract of the pig and all produce eggs which are more or less alike. Any conclusions therefore based on egg counts and on larvæ hatched from eggs of indefinitely known origin, must be regarded with suspicion. Thus the figures of Ackert and Payne which they based on the examination of droppings alone, cannot be accepted as conclusive. This is also true with regard to the results of their infection experiments with larvæ hatched from the droppings of Trinidad pigs. There is no evidence that in these cases they were dealing with Pure Necator infections; in fact, it is almost certain that they were not. It is true that the larvæ of *Œsophagostomum dentatum* can be easily distinguished from that of Necator—Ackert and Payne point out that they discovered undetermined nematodes in the large intestine of pigs after an experimental infection with cultures of hookworm larvæ contaminated by the larvæ of this nematode. But there is no information as to the morphology or bionomics of Globocephalus. It can be assumed, however, that it will resemble somewhat closely the other hookworm larvæ and will be distinguishable only with some difficulty. Their figures of the distribution

of *Necator suillus* and their conclusions based on the larvæ will accordingly require revision.

Ascaris lumbricoides is present in human beings in the West Indies to the extent of about 60 per cent.; in fact, it is the most prevalent human helminth in many of the islands. In over two hundred pigs examined I found Ascaris in only two of them—once in Trinidad and once in St. Kitts—and in each a single specimen was recovered. The American Hookworm Commission was rather more fortunate, however, and found this parasite in as much as 10·8 per cent. of the pigs in certain districts in Trinidad. Pigs throughout these islands are given every facility to ingest human excrement and become infected, and the adverse climatological conditions discussed earlier in this article, as is shewn by the high human infection in islands where the hookworm infection is very low, do not hold good in the case of Ascaris. The fact that the human Ascarids do not develop in pigs suggests very strongly that two biological varieties of the parasite occur—one human, the other porcine.

Trichocephalus is, like Ascaris, an uncommon parasite in pigs in the West Indies, although it also is a common human parasite. Possibly in this case also, biological varieties exist.

Metastrongylus elongatus and *M. brevivaginatus* are both found not uncommonly in the lungs.

Macracanthorhynchus hirudinaceus also occurs in the small intestine of the pig, and at first sight it may be mistaken for an Ascaris. On several occasions actual perforation of the intestine was observed.

Horses, Donkeys and Mules.

These animals shew the usual great variety of parasites and are probably more highly infected than any of the other domestic animals. However, the opportunities for examining them were few and the list of parasites found is consequently small. There are no obvious differences in their parasitic fauna from that found in equine hosts in other warm countries;

and as usual the Sclerostomes and Cylichnostomes were the commonest species found, although the insect-borne forms are also fairly common.

PIGS.

Moniezia expansa	accidental.
Cysticercus cellulosæ	not very common.
Ascaris lumbricoides	accidental.
Trichocephalus trichiurus	rare.
Stephanurus dentatus	very common.
Necator suillus	common.
Globocephalus urosubulatus	common
Œsophagostomum dentatum	common.
Hyostrongylus rubidus	common.
Metastrongylus elongatus	common.
M. brevivaginatus	common.
Macracanthorhynchus hirudinaceus ...	not uncommon.

SHEEP AND GOATS.

Fasciola hepatica	not common.
Moniezia benedeni	not very common.
M. expansa	not very common.
Cysticercus tenuicollis	not uncommon.
Trichocephalus ovis	not uncommon.
Monodontus trigonocephalus	not uncommon.
Chabertia ovina	not common.
Œsophagostumum columbianum ...	not common.
O. venulosum	not common.
Hæmonchus contortus	not very common.
Trichostrongylus instabilis	very common.
T. vitrinus	not common.
Cooperia curticei	not common.
C. oncophora	not common.
Ostertagia circumcincta	not common.
Dictyocaulus filaria	not common.
Muellerius capillaris	fairly common.

Cattle.

Paramphistome species (*)	uncommon.
Cysticercus bovis	uncommon.
Ascaris species (*)	uncommon.
Setaria labiato-papillosa	not uncommon.
Monodontus phlebotomus	uncommon.
Œsophagostomum radiatum	not uncommon.
Dictyocaulus viviparus	not common.

Equines.

Ascaris equorum	common.
Oxyuris equi (*)	not common.
Habronema microstoma	not uncommon.
H. muscæ (*)	not uncommon.
Setaria equina (*)	not uncommon.
Strongylus vulgaris	common.
S. edentatus	common.
S. equinus	common.
Triodontophorus minor	not uncommon.
T. serratus	not uncommon.
Trichonema spp.	very common.

(*) These species were not found by the writer, but their presence was reported by the local veterinary authorities.

Conclusion.

There were no new species seen among the helminth parasites of stock in the West Indies. As usual the degree of infection is proportional to the concentration and lack of movement of the hosts. Climatic and geological conditions are not very favourable for the development of parasites and, accordingly, infections, although common, are generally light. Species having a free-living larval stage, are especially so.

Important species from the economic point of view are the cysticerci in pigs and cattle of the two human tænias—both relatively uncommon—and the kidney-worm of pigs. It is impossible yet to estimate accurately

the importance of *Necator suillus* in connection with human infection; but it is doubtful if it has any real significance. A point of interest is the great preponderance among the smaller ruminants of *Trichostrongylus instabilis* and the complete absence of Nematodirus. The most important parasitic diseases of stock in the West Indies, however, are not helminthic, but protozoal, particularly those, such as Babesiosis, which are carried by arthropods.

References.

Ackert, J. E., and Payne, F. K., 1923.—" Investigation on the Control of Hookworm Disease, xii.," *Amer. J. Hyg.* iii., pp. 1-25. (*W.L.* 600a.)

Cameron, T. W. M., 1929.—" Observations on a Parasitological Tour of the Lesser Antilles," *Proc. R. Soc. Med.*, xxii., pp. 933-41. (*W.L.* 16916.)

Leiper, R. T., 1913.—" Observations on Certain Helminths of Man," *Trans. Soc. Trop. Med. Hyg. Lond.*, vi., pp. 265-297. (*W.L.* 21671.)

Ransom, B. H., 1911.—"The Nematodes parasitic in the alimentary tract of Cattle, Sheep and other Ruminants," *Bull. U.S. Bur. Anim. Ind.*, 127. (*W.L.* 5642.)

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp.* 85-88.

On the Presence of Fats in the intestinal wall of Nematodes.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

In November, 1926, the writer observed the active revival of larvæ of *Tylenchus dipsaci* in water after being kept in a dry condition since September, 1920 ; a period of just over 6 years. These almost adult larvæ, which had been collected from Narcissus bulbs, were richly supplied with an abundance of reserve food bodies in the wall of the intestine and an attempt was made to determine the nature of these reserves. Various tests were carried out with different chemical reagents and stains and it was finally found that they were composed of fats in the form of small, spherical droplets of oil of variable size. Following these observations, as opportunity arose, other plant-parasitic and free-living nematodes have been examined for the presence of fats in and around the intestinal wall.

A study of the literature dealing with nematodes revealed the fact that fats have been recognised in the intestinal wall by some early investigators and also by recent workers as the following quotations show.

Davaine (1857), p. 25, writes of the presence of an albumino-fatty substance in the intestine of *Tylenchus tritici*.

Bastian (1865), p. 81, writing on the intestine of nematodes, says : " It is made up of a central tube and a mesenteric envelope, between which is situated a uniform layer of cells containing light or olive-coloured fat particles probably having a rudimentary hepatic function."

The same author (1866), p. 581, says : " I have alluded to the occasional large quantity of fat met with within the alimentary canal of these animals, apparently as a primary product in the process of assimilation."

Perez (1866), p. 50, in dealing with the intestine of *Rhabditis terricola* Dj., speaks of the presence of granulations composed of an albumino-fatty

substance or simply of fats. He compares them with the larger concretions found in the intestine of the vinegar eelworm, *Anguillula aceti*, in which he recognises their fatty character.

de Man (1884), p. 21, writing on the anatomy of free-living soil nematodes, says " Der Darm ist häufig mit blasen Fettkügelchen angefüllt, welche bisweilen, wie bei einigen Dorylaimen, eine verschiedene Farbe besitzen und gelblich oder röthlich aussehen können."

Payne (1923) investigated the migratory movements of mature infective larvæ of *Necator americanus* and studied the nature of the reserve food granules stored in the intestinal cells and in the body-cavity in the region of the œsophagus. By staining with Sudan III she found that there were two kinds of granules present, large ones which took the stain and are fatty in character and smaller ones which remain unstained by this reagent.

Peters (1927), in his account of the anatomy of the Vinegar eelworm, *Turbatrix* (*Anguillula*) *aceti*, quotes Henneberg's opinion that the abundant round globules in and around the intestinal wall, are fatty in character and describes his own tests with Scharlach R. and Nile blue whereby he proved that they are composed of neutral fats.

In Bolles Lee (1921), pp. 356-369, detailed methods are given for the detection of fats and substances of a lipoid nature. The chief of these are the fat stains, Sudan III, Scharlach R, and Nile blue, blackening by Osmic acid solubility in various fat solvents and behaviour in polarised light. The writer has most frequently used Scharlach R. and Nile blue ; the former staining fats bright red and the latter staining neutral fats pink, fatty acids blue and mixtures of the two pinkish blue.

The usual technique adopted has been as follows : the worms to be tested are collected by a capillary pipette into a small drop of water in a glass capsule. They are then fixed in hot 70 per cent. alcohol, containing about 2 per cent. glycerine, which is poured on to them. A drop of Scharlach R, or Nile blue, made up in the appropriate strength, is then added and the capsule is set aside to allow the alcohol to evaporate. The worms are finally left in weak glycerine and show the fat droplets stained within the body. If mounted on slides in the glycerine in which they have been stained, those treated with Scharlach R. keep the red colour well for many months, whilst those stained with Nile blue lose colour after a few weeks.

Results.

Fats have been demonstrated by the use of Scharlach R. in the form of small droplets of oil in the intestinal wall of the following species of nematodes in which they are frequently abundant and must be regarded as one of the principal products of digestive metabolism. *Rhabditis aspera*, *R. pseudoxycerca*, *R. monhystera*, *Cephalobus rigidus*, *C. persegnis*, *Dorylaimus longicaudatus*, *Plectus granulosus*, *Diplogaster longicauda*, *Myolaimus heterurus*, *Aphelenchus parietinus*, *A. fragariæ*, *Tylenchus robustus*, *T. dubius*, *T. pratensis*, *T. intermedius*, *T. musicola*, *T. hordei* and *T. dipsaci*. In the second stage resistant larvæ of *T. tritici* and *T. graminis* they are probably to be regarded as derived from the fats of the egg yolk.

They have been found in the following species which are predatory carnivorous forms: *Mononchus muscorum*, *M. tridentatus* and *Diplogaster vorax*, and it is feasible to assume that in these, they are obtained from the fats of the devoured nematodes.

The only free-living saphrophytic nematode in the intestinal wall of which fats have not been discovered is *Cheilobus quadrilabiatus*. In this species, females only of which are known, the cells of the intestine are filled with large granules of a dark grey colour, which do not give any of the fat reactions with stains. When worms mounted in water are killed by heat, or when treated with dilute acetic acid, the granules slowly disappear and leave a faint refractive outline of their original shape. They do not stain with iodine or neutral red and the writer has been unable to determine their nature. Fats are present in the eggs of this species as has been revealed by Osmic acid, Sudan III and Scharlach R.

In the intestinal wall of *Tylenchus hordei* obtained from galls on the roots of *Elymus arenarius* and *Poa pratensis*, oil droplets were found to be very abundant and staining with Nile blue showed that they are mainly composed of neutral fats staining pink with admixtures of fatty acids, giving a bluish tint, here and there, to the pink droplets.

The pre-adult resistant larvæ of *Tylenchus dipsaci*, which sometimes issue in enormous numbers from the sides of the basal plate of diseased narcissus bulbs when the latter are dried, are extremely rich in reserve fats. The droplets stain with Nile blue and are chiefly neutral fats

mixed with fatty acids. Lying between the large oil droplets are certain much smaller rounded granules which stain with neutral red, at least in dead specimens of the worm; in living examples the stain does not seem to penetrate at all. What their composition is the writer cannot say. They do not stain with iodine and consequently are probably not composed of glycogen.

The fats occur as droplets of oil within the cells of the intestine, in the body-cavity around the œsophagus and in the tail region. They are composed of non-drying oils as was proved by crushing several dried worms on a slide when the oils were left on the glass as small drops. The slide was then exposed to the air in a Petri-dish for about 6 weeks. The drops kept the same appearance throughout, showing no sign of drying or of the formation of a skin on the surface. It may be concluded from this that they are the fats of saturated fatty acids or at any rate are the esters of such an unsaturated fatty acid as oleic acid which, according to Leathes and Raper (1925) is of widespread occurrence in animal tissues.

At the present time it is not possible to carry further the determination of the composition of the fats and fatty acids occurring in nematodes. The quantities of material available are so small that one must be content, for the present, with the demonstration of their presence by stains, etc., and with the recording of the non-drying character of the oil droplets.

References.

Bastian, C. H., 1865.—" A Monograph of the Anguillulidæ," *Trans. Linn. Soc.*, vol. 25.

— 1866.—" Anatomy and Physiology of the Nematoids, Parasitic and free, etc.", *Phil. Trans. Roy. Soc.*

Davaine, C., 1857.—" Recherches sur l'Anguillule du blé niellé, etc.", Paris.

Leathes, J. B., and Raper, H. S., 1925—" The Fats," 2nd. ed., London.

Lee.—" The Microtomist's Vade-Mecum," 8th ed., London.

Mann, J. G. de, 1884.—" Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der niederländischen Fauna." Leiden.

Payne, F. K., 1923.—" Investigations on the control of hookworm disease, xxx. Studies on factors involved in migration of hookworm larvæ in soil," *Am. J. Hyg.*, vol. iii, No. 5, pp. 547-583.

Perez, J., 1866.—" Recherches sur l'Anguillule terrestre," Paris.

Peters, B. G., 1927.—" On the Anatomy of the Vinegar Eelworm," *Jl. Helm.*, vol. v, No. 4, pp. 183-202.

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp*. 89-92.

A new species of the nematode genus *Cylindrogaster*.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine*).

The writer established the genus *Cylindrogaster* in 1927 for the reception of a nematode found in cultures of rat's fæces. Its principal distinguishing features were the possession of very long buccal rods and an elongate, cylindrical, muscular first œsophageal bulb. The male possessed 10 caudal papillæ arranged in three groups disposed in a manner exactly similar to that of the caudal papilæ in many species of *Diplogaster*, *Odontopharynx longicaudata* and *Tylopharynx striata*.

Stefanski (1928) published a short paper in which he pointed out that a species of nematode, which he had described in 1922, under the name of *Rhabditis longistoma*, was most probably the same as the writer's *Cylindrogaster coprophaga*. He agreed that the worms should be placed in the genus *Cylindrogaster* but suggested, on grounds of priority, that his specific name *longistoma* should replace the writer's name *coprophaga*.

The purpose of the present communication is the description of another species having œsophageal characters typical of the genus but the male possessing a bursa-like expansion of the tail with papillae supporting it in the manner frequently found in males of the genus *Rhabditis*. In the female also the gonad is single and not double as in *Cylindrogaster longistoma*.

The new worms were obtained at Winches Farm, St. Albans, from débris taken from beneath the bark of a fallen elm tree riddled with tunnels made by bark-boring bettles. Some of the moist dark powdery material was extracted in cold water by the Baermann method and the

worms were found in searching through the water drawn off from the funnel after two or three hours.

Morphology.

Principal measurements :—Female, length, 0·78 mm. to 1·1 mm. ; breadth, 0·03 mm. to 0·06 mm. ; head to end of œsophagus, 0·18 mm. to 0·2 mm. ; head to vulva, 0·45 mm. to 0·64 mm. ; anus to tip of tail, 0·08 mm. to 0·12 mm. Male, length, 0·77 mm. to 0·98 mm. ; breadth, 0·03 mm. to 0·05 mm. ; head to end of œsophagus, 0·18 mm. to 0·2 mm. ; anus to end of bursa, 0·034 mm. ; spicules, 0·048 mm. ; gubernaculum, 0·018 mm.

Proportions :—Female, α=18·3 to 26·5 ; β=3·6 to 5·2 ; γ=8·9 to 10·2. Male, α=17 to 26 ; β=4·2 to 5.4 ; γ=22·6 to 28·8.

The adults of both sexes are stouter in build than those of *Cylindrogaster longistoma*. The excretory pore, as in that species, is very difficult to distinguish in freshly killed specimens. The head characters are the same as those of *C. longistoma* viz., six roundly conical lips each bearing a very small papilla. There is a highly refractive cuticular spot on either side of the mouth opening separated by a short break from the beginnings of the buccal rods. The latter measure 0·04 mm. to 0·05 mm. long, which is practically the same as in *C. longistoma*. The cylindrical muscular bulb is 0·06 mm. to 0·07 mm. long by 0·02 mm. in median width. It is slightly broader posteriorly than anteriorly. In the substance of the bulb there are longitudinal supporting strands, the anterior ends of which are curved inwards towards the lumen. At about two-thirds down the bulb the strands swell out a little and after running parallel to the lumen turn in towards it at the end of the bulb as shown in fig. 1.

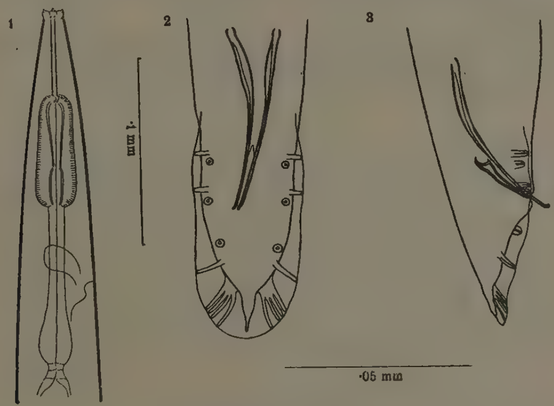
The second bulb of the œsophagus is a comparatively small swelling not so wide as that in *C. longistoma* and, as in that species, a valve apparatus is not found in it.

The intestine in most of the specimens examined contained a quantity of short fungal hyphæ which probably serves as a source of food since though separate elements could be distinguished at the beginning of the intestine there was generally only an amorphous mass at the rectal end.

Female.—The vulva is situated a little behind the middle of the body. The gonad is single and anterior in position. It is reflexed on itself and

the ovary frequently extends for a short distance posterior to the vulva. There is, as a rule, one egg at a time in the uterus. A short pointed post-vulval uterine sac is present. The tail tapers evenly to a sharp point but is not long and filiform as in *C. longistoma*.

Male.—The gonad is single, and, as in *C. longistoma*, reaches about two-thirds the distance from anus to the end of the œsophagus. Its anterior end is reflexed for a short distance. The spicules are long and slender. Each is slightly expanded anteriorly into a rather small open head, whilst the points are narrow but rounded and have the tips tilted forward a little as shown in fig. 3. The bursal membrane surrounds



Cylindrogaster ulmi n. sp.

Fig. 1.—Oesophageal region in lateral view.
Figs. 2 and 3.—Male tail in ventral and lateral aspect.

the tip of the tail. There are 10 supporting caudal papillæ on each side arranged as shown in figs 2 and 3. Four of these are pre-anal and 6 post-anal in position. Of the pre-anal ones, the most anteriorly placed is directed laterally and reaches to the edge of the bursa. It is immediately followed by one which points ventro-laterally. This arrangement is repeated in the next two papillæ. Post-anally there is first a ventro-laterally placed papilla, situated about half-way between the anus and the base of the terminal tail process. A short distance behind this there

is a rather long papilla, dorso-laterally directed, reaching to the edge of the bursal membrane. Posterior to this there is a compact group of three slender papillæ lying very close together with a fourth one arising from the same base but directed a little more dorsally than the group of three.

Specific name.—Since the worms were obtained from the bark of an elm tree, the new species has been named *Cylindrogaster ulmi*.

References.

Goodey, T., 1927.—" *Cylindrogaster coprophaga* gen. et sp. nov., A Nematode found in a Culture of Fæces from a Wild Brown Rat," *Jl. Helm.*, vol. v, No. 1, pp. 25-32.

Stefanski, W., 1928.—" Sur l'identité des espèces *Rhabditis longistoma* Stefanski, 1922 et *Cylindrogaster coprophaga* Goodey, 1927," *Jl. Helm.*, vol. vi, No. 2, pp. 77-78.

Journal of Helminthology, Vol. viii., *No.* 2, *June,* 1930, *pp.* 93-102.

Histological Observations on Experimental Ascariasis

By William K. Blackie, M.B., Ch.B., D.T.M., & H.

(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Introduction.

The lesions associated with the migration of the larvæ of *Ascaris megalocephala* through the tissues of the host were studied in rabbits, guinea pigs and mice. In addition to studying the changes in those organs directly invaded by the larvæ in the course of migration, an organ remote from the migratory route but exposed to the action of any toxic products elaborated by the larvæ was also examined. In each case therefore, sections were prepared from (a) liver, (b) lungs and (c) kidneys.

Many thousands of embryonated eggs of *Ascaris megalocephala* were administered to each experimental animal ; the actual dosage varying directly as the size of the animal. In each case the animal died or was killed three to seven days from the time of the infective feed. When necessary, the animal was killed by first stunning and then incising the throat. Gas was not employed for this purpose on account of the possibility of its contributing to the production of pulmonary lesions. A post-mortem examination was carried out on each animal within an hour of death.

Systematic examination of the tissues already mentioned revealed the fact that the essential lesions were alike in all three species of experimental animal. The subsequent description of the pathological changes associated with the migrations of the ascaris larvæ will apply therefore

to any of the three species of animals and whatever differences may exist are mainly differences in the degree or extent of the lesions.

Pathological Changes in the Liver.

The most salient feature of the sections of the liver is the damage to the hepatic parenchyma. All gradations from mere cloudy swelling to extensive necrosis of hepatic cells may be encountered, depending on the duration and severity of the infestation. Although the liver is diffusely involved the degenerative changes are always most marked around the intra-lobular vein whence they extend out towards the periphery of the lobule, which in the very early phases of the disease is relatively slightly affected. Even in those animals dying of the disease it was found that the most profound necrosis occurred in the centre of the lobule. The affected cells of the central zone may consist simply of an indistinct nucleus, a cell membrane and little else or where the necrosis is less severe the cells show loss of definition, the nucleus stains faintly (sometimes so faintly as to be almost invisible) while there is considerable loss of cell protoplasm. Towards the periphery of the lobule the hepatic cells will generally be found to be less severely damaged, but in all the animals examined they showed severe cloudy swelling without actual necrosis and loss of protoplasm. There is no sharp line of demarcation between the severely and less severely damaged zones, although with low magnifications the transition from one to the other may appear relatively abrupt. In the severely damaged parts of the lobule the endothelial cells of the hepatic sinusoids and the Kupffer cells stand out prominently.

Where an animal died it was impossible to demonstrate a single normal liver cell in any of the sections examined.

In the rabbit it was found that in the centre of the lobules where necrosis was most severe, the defects in the liver trabeculæ had been filled by the distended blood sinusoids. In some cases the wall of the sinusoid had apparently given way with the production of an area of hæmorrhage of greater or less extent. Thus in a rabbit which died on the fifth day of the disease, there was not only extensive necrosis of liver parenchyma but also widespread hæmorrhages throughout the lobules.

Another striking feature, particularly in the case of the guinea pigs,

is the presence of cell clusters scattered throughout the liver substance. These small clusters occur mainly at the periphery of a lobule frequently in relation to a small bile duct. The predominating cell is the eosinophile leucocyte which constitutes 75 per cent. or more of the total cells present in the granuloma. Cells of the mononuclear type, together with an occasional neutrophile polymorph are also found in these foci. It is common also to find portions of residual cytoplasm and an occasional connective tissue strand mingled with the cell cluster suggesting that the cluster has been formed at the expense of the liver cells in the immediate vicinity. On the other hand it is noteworthy that the liver cells

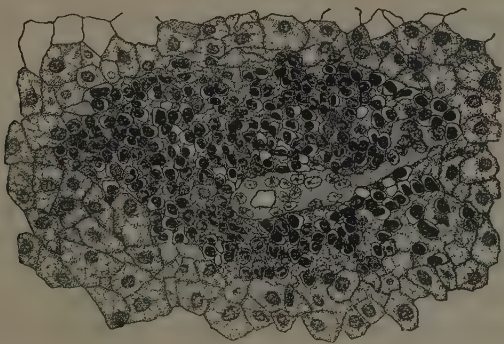


Fig. 1.—An example of the focal accumulation of eosinophile leucocytes and mononuclear cells in the liver substance.

on the immediate periphery of the granulomata are generally found to be in a better state of preservation than liver cells elsewhere. In addition to occurring in the form of granulomata the eosinophile leucocyte is also encountered either singly or in small groups scattered throughout the whole of the liver tissue, while in the blood vessels in the sections the eosinophile leucocyte is the predominating white cell.

Very occasionally a larva can be demonstrated in the midst of a cell-cluster, but it is more usual to find that the tissues in the immediate vicinity of the larvæ have reacted to only a slight degree. The immediate reaction usually takes the form of a scanty collection of cells in close

proximity to the larva. The eosinophile leucocyte is the cell chiefly concerned, but they are frequently found to show degenerative changes. The nucleus tends to break up or become vesicular, the eosinophile granules are small and scanty and in many cases the cytoplasm of the cells appears completely disintegrated with eosinophile granules lying free.

Remains of the liver cells may also be found in the immediate neighbourhood of the larvæ together with some connective tissue strands derived from the supporting framework of the liver. Mononuclear cells are also met with.

The intense eosinophilic infiltration already described is only rarely present in relation to a larva and indeed it is by no means uncommon to find a larva lying within a liver sinusoid without inducing any local cell response whatever.

Pathological Changes in the Lungs.

Areas of intra-alveolar hæmorrhage are found throughout the whole of the lung but the extent of the hæmorrhage varies considerably in different areas. In some parts the alveoli are completely filled with blood, in other parts where the bleeding has been less, air has entered the alveoli and forced the blood up against the alveolar walls thus conferring a coarse appearance upon the delicate alveolar network. In still other areas the pulmonary capillaries are deeply engorged but intra-alveolar hæmorrhage is not present.

The alveolar content also varies in character. In some parts it consists of blood only, indicative of recent hæmorrhage, but more commonly in addition to blood cells there are numbers of large mononuclear cells present in the exudate. These large mononuclear cells are actively phagocytic. In a few instances it was possible to demonstrate an intact red blood cell within the cytoplasm of the mononuclear. On the other hand practically every cell of this nature contains within its cytoplasm a finely granular dark brown iron-containing pigment which is presumably derived from ingested red blood cells. In some cases the cell is so heavily pigmented that the nucleus is completely obscured. The distribution of these pigmented mononuclear cells is more or less co-extensive with the intra-alveolar hæmorrhage, consequently there is abundant evidence of phagocytosis in all such areas. While

on the one hand some alveoli contain only blood, on the other hand some alveoli contain an exudate of pigment-bearing mononuclears representing the sequel to intra-alveolar hæmorrhage. These cells may still be discrete and well defined or they may exhibit degenerative changes. Another type of phagocyte is present in the shape of a large multi-nucleated cell which appears to be formed by fusion of a number of the mononuclear cells already described. These large cells show considerable variation in size, shape and in the number and staining reaction of the nuclei, but in all cases they are heavily charged with dark-brown pigment.

Fig. 2.—A pulmonary alveolus containing a number of pigment-bearing mononuclear cells and showing three examples of multinucleated phagocytes.

In addition to intra-alveolar hæmorrhage, there is in all cases a greater or less amount of œdematous exudate. Where the hæmorrhage is severe the œdema tends to be masked, but where the alveoli contain few cells the œdema is readily recognized and frequently extends out beyond the areas of hæmorrhage and out into the bronchioles.

As in the liver, so in the lung cell clusters may be encountered. They are a specially prominent feature of the sections of rabbit lung. Eosinophiles occur in variable numbers in these foci while small round cells and a larger cell with a faintly staining oval nucleus and abundant cytoplasm also share in their formation. Hæmorrhage within these foci is also common, while the presence of a variable quantity of nuclear débris indicates some degree of cell necrosis. In addition to being aggregated into foci the eosinophile leucocytes also occur singly or in small numbers through the lung tissue generally. Larvæ can be easily

recognised. They may occur in the wall of an alveolus or free in an alveolar space, where they are usually associated with a certain amount of blood. They may also be found in the midst of a cell cluster. When they occur within an alveolus containing a large number of pigmented mononuclears, the larvæ frequently appear to be undergoing dissolution, as suggested by the fact that the outline of the larvæ lacks sharp definition and the nuclei stain faintly.

The majority of the bronchioles in relation to the areas of hæmorrhage contain a certain amount of exudate. The exudate may consist entirely of blood although more often it consists of red blood cells, an occasional intact mononuclear together with a large amount of indeterminate granular débris. The exudate is not as a rule copious, although complete plugging of a bronchiole is occasionally met with. As already stated fluid exudate may extend out into the bronchioles from the alveoli. Some degree of cloudy swelling is sometimes present in the bronchiole epithelium.

Pathological Changes in the Kidneys.

The kidney is diffusely involved but the extent of the involvement varies in the different subdivisions of the renal unit.

The glomeruli are mostly congested and in some instances a trace of exudate (usually granular) is present between the surface of the glomerulus and Bowman's capsule. The endothelial cells that line the glomerular space may sometimes be unduly prominent and in some cases have actually desquamated.

The chief pathological change is found in the cells of renal tubules and the portion of the tubule most affected is that part which lies between the loop of Henle and the end of the junctional tubule. Between the glomerulus and the loop of Henle the tubular epithelium shows only a moderate degree of cloudy swelling. Distal to this point, the cells of the tubules are very extensively necrosed. The free ends of the cells are ragged while the cytoplasm is highly granular and deficient in amount. The cells may be almost entirely disintegrated with only a faintly staining nucleus and some residual cytoplasm to mark their position. As a consequence of this necrosis, the lumina of the affected tubules contain accumulations of cell débris and in some cases cast formation has occurred.

No definite changes are to be observed in the interstitial tissue. Although the kidney as a whole is congested no changes are present in the blood vessels. Furthermore, no larvæ were demonstrated in the kidney substance.

Commentary.

While the lesions described above have been produced as a result of relatively massive doses of embryonated ascaris eggs, there is no reason to suppose that they differ from those arising out of natural infection other than in the matter of degree. Large doses were employed in order to produce well defined pathological changes and so facilitate a comparison of the relative importance of the various lesions in the tissues examined.

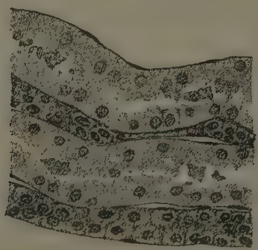


Fig. 3.—Renal tubules showing necrosis of ephithelium with less affected portions of tubule alongside.

The two factors concerned in the production of the lesions are (*a*) the mechanical action of the larvæ and (*b*) their toxic products, but while the mechanical effects are largely confined to vascular endothelium and capillary walls in the migratory route, the toxins of the larva pass into the circulation and are carried to all parts of the body with the result that toxic changes occur in organs that are not exposed to larval migration.

As the ascaris larvæ reach the liver via the portal vessels and as the larvæ can reach the lung only by first traversing that organ, both mechanical and toxic effects are likely to be more pronounced in the liver than elsewhere. The larvæ as it were are focussed on the liver whereas they tend to be diffused over the lungs. From the description of the

pathological changes given above it is evident that widespread degeneration occurs in the hepatic parenchyma. The nature and extent of the degeneration are such as cannot be accounted for merely by the mechanical presence of ascaris larvæ. It becomes necessary therefore, to postulate some toxin elaborated by the larvæ and producing degenerative changes in the hepatic epithelium in the nature of cloudy swelling and actual necrosis. The liver cells appear to undergo a process of lysis with marked loss of cytoplasm and nuclear degeneration. Further indirect evidence of the presence of a larval toxin is found in the cell clusters scattered throughout the liver substance. On investigating the cells concerned in the production of these foci, it was found that the eosinophile leucocyte predominated and as helminthic toxins have been shown to exert a positive chemiotactic influence on these cells, it follows that their presence in such large numbers in these foci supports the theory of larval toxin production.

More conclusive evidence of the presence of a larval toxin is to be found in the demonstration of immune body in the serum of rabbits during the stage of larval migration. It is of interest to note that it is more the exception than the rule to encounter larvæ in the midst of these cell clusters, suggesting that the local infiltration occurs in the wake of the migrating larva rather than immediately around it.

Perhaps the most striking feature of the hepatic lesions is the extent of the degenerative changes. In the animals exposed to heavy infections and in those dying of the disease it was not possible to demonstrate a single normal liver cell in any of the sections examined. It is evident therefore, that hepatic insufficiency must be an important contributory factor in the cause of death.

While in the liver the important pathological changes are toxic in origin, in the lung the mechanical effects of the larvæ tend to overshadow the toxic changes. By the time the larvæ reach the lungs they have increased considerably in size and being unable to pass the pulmonary capillaries they break through into the lumen of an alveolus. It is possible however that the rupture of the capillary wall may be facilitated by the larval toxin. The presence of blood causes the alveolar endothelium to swell up and become free in the lumen of the alveolus. These endothelial cells are actively phagocytic and rapidly ingest the red blood cells within the alveoli, so that in a short time, the lung tissue

is overrun with heavily pigmented mononuclear cells—the pigment having been derived from ingested erythrocytes.

The ultimate fate of the pigmented cells was not determined but they probably pass out into the bronchioles and up the respiratory tract or enter the pulmonary lymphatics and finally come to rest in the lymph glands of the hilum where the blood pigment will be deposited. The large multinucleated phagocytic cells found from time to time are probably formed by fusion of the mononuclear cells derived from the alveolar endothelium.

As indicated above the intra-alveolar hæmorrhage and the evidences of phagocytosis tend to obscure the toxic manifestations. Small cell clusters containing numerous eosinophiles occur in the lung but to a less extent than in the liver, and in addition eosinophiles are found scattered more or less diffusely throughout the lung tissue. The eosinophilic response taken in conjunction with the generalised congestion and the œdematous patches in the lung may be regarded as a reaction to the larval toxin. The exudate within the bronchi appears to be mainly of alveolar origin.

Broncho- or lobar-pneumonia in the true pathological sense cannot be regarded as a characteristic of ascaris invasion of the lung. On the other hand a lung damaged in the manner described is likely to be highly susceptible to bacterial invasion and in such an event the host tissues, already affected by the ascaris toxin will be less able to react adequately to any additional toxin.

Since the kidney is remote from the migratory route of the ascaris larvæ, any pathological changes occurring in its substance may be regarded as purely toxic in origin. The principal lesion consists in necrosis of the epithelium of the renal tubules particularly in that portion of the tubule where the glomerular filtrate is undergoing or has undergone concentration, with cloudy swelling elsewhere.

The glomerulus is also involved to a variable extent but apparently rarely sustains serious damage. The renal lesion may be described therefore as an acute toxic nephritis affecting the renal tissue more or less uniformly and caused by some toxic substance present in the circulation. The toxic substance probably originates in the ascaris larvæ, although the breaking down of liver tissue may be a further source of toxic bodies. The diffuse character of the lesion must necessarily

interfere with renal function to a greater or less extent and thus impair the efficacy of the general host reaction.

Conclusions.

1. The lesions associated with experimental ascariasis may be ascribed (a) to the mechanical action of the migratory larvæ, and (b) to toxic substances elaborated by the larvæ.

2. The larval toxin has a destructive effect on specialised epithelium, hence the liver and kidneys show evidence of diffuse and often severe toxic necrosis.

3. The pulmonary changes consist essentially of intra-alveolar hæmorrhage with a greater or less amount of œdema.

4. The cause of death in fatal cases of the disease is to be ascribed to profound toxæmia associated with extensive hepatic and renal lesions rather than to pulmonary involvement which, though a contributory factor, is apparently only of secondary importance in causing the death of the animal.

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp.* 103-122.

Field Studies on *Heterodera schachtii* Schmidt in relation to the Pathological Condition known as "Potato Sickness."

By Herbert W. Miles, M.Sc. (Bristol), N.D.A.

(Adviser in Entomology, Victoria University of Manchester).

Preliminary studies in Lancashire and Cheshire (Smith and Prentice, 1929) have indicated that under certain conditions there is a positive association between the disease known as "potato sickness" and the intensity of root eelworm, *Heterodera schachtii* Schmidt, as measured by the cyst content of the soil. This association does not appear to be constant, as a moderately high cyst content may occur in the absence of obvious disease. It has also been found (Smith and Miles, 1929) that under conditions prevailing in the North West of England the cyst content of the soil increases only after a crop which is not a total failure.

In the work of Morgan (1925 and 1926) the same conflicting evidence of the relation of *H. schachtii* to "potato sickness" is apparent. In the course of his investigations Morgan found that in Lincolnshire, certain areas where plants were doing well had a cyst content similar to that of areas where pathological condition in potato plants was most pronounced.

Hitherto most of the work on the relation of *H. schachtii* to disease in potatoes has been based on a study of the cyst content of the soil, and the influence of soil reaction on cyst content. In the recent work of Morgan and Peters (1929), however, an effort is made to associate the pathological condition of the plants with cyst content and soil reaction, but no conclusions are drawn.

The widespread occurrence of "potato sickness" in certain areas of Lancashire and Cheshire during recent years has led to insistent demands for further study of the relationship between the presence of *H. schachtii*

and the incidence of diseased conditions in potato crops. Since there appears to be no published account of the life history of *H. schachtii* on potatoes under English conditions, it was felt that at the outset definite knowledge of the organism was essential. With this knowledge it was hoped to correlate the development of the nematode in the plant tissue with the pathological conditions of the host plant.

In order to study the development of the eelworm, potato plants were grown on land known to have produced a diseased crop the preceding year. The area selected produced a yield of about 30 cwt. per acre in 1928. The soil was carefully sampled and the cyst content determined, using the method described by Morgan (1925). Ten cyst counts were made from the composite sample, each count being made from 10 grammes of air dried soil. The cyst counts were as follows : 30, 28, 37, 21, 28, 27, 17, 26, 26 and 28, the average being 26·8. The land received the normal cultural treatment in the autumn and spring, and was manured with stable manure, according to the usual practice on the holding. Potatoes of the variety " Great Scot " were planted in May.

At weekly intervals plants from this plot were examined in the laboratory. The roots were carefully washed in running water, and 5 mm. lengths were selected for microscopic examination. The eelworms were dissected out from these lengths and identified. In this way information regarding the development of *H. schachtii* in the tissue of the potato plant was obtained.

Life History of *H. schachtii* on Potatoes.

Examination of potato plants showed that first stage larvae of *H. schachtii* gain admittance to the plants within a few days after the formation of roots, which develop on the setts in a week to a fortnight after planting, depending upon the temperature and moisture conditions of the soil. In the first instance some few young larvæ may be found in the primary roots, but it is not until the formation of secondary roots that the main invasion of first stage eelworm larvæ takes place. As a result of some hundreds of dissections, it would appear that the eelworms enter near the tips of the secondary or, later in the season, tertiary rootlets, and travel upwards towards the older tissue, following the course of the endodermis but remaining on the outside of the pericyclic tissue.

After a short time the first ecdysis occurs. The position in which the young second stage larvæ occur most abundantly is the region of the origin of the lateral roots. They occur in the cortical tissue of both main and lateral roots, usually with the head embedded in the endodermal tissue. Occasionally the larvæ undergo the first ecdysis in the lateral roots some distance from their origin, but this is not usual.

Under normal conditions development is rapid, and about a week later a second ecdysis takes place. At this stage the males, which are elongate, can be readily distinguished from the pyriform females. The females have their heads embedded in the endodermal tissue, and their rapidly swelling bodies are gradually pushed between the cortical cells until the posterior portion protrudes through the epidermis. The males undergo a final ecdysis at or near the surface of the root and escape to the exterior of the rootlets. Female eelworms appeared more numerous than the males during the course of these investigations, and a series of counts gave 214 males to 590 females, a proportion of 1 : 2·75.

Mating takes place on the exterior of the roots. Although this was not observed by the writer, a large number of dissections indicated that it occurred between the time of the first rupturing of the root surface tissue and the complete protrusion of the body of the female. The ovaries develop fairly rapidly and egg outlines soon become visible. In a comparatively short time the outlines of the embryo can be discerned, and the complete development of the embryo, sometimes followed by the eclosion and escape of young larvæ, occurs during the same season. In no case has the writer observed the presence of an " egg-sac " such as that described by Strubell (1888) on the beet strain of *H. schachtii*, and mentioned by Thorn (1926) on beet eelworm in America.

Observations on the occurrence of the various stages showed that first stage larvæ were present in the root tissue until August 6th, the only factor which appeared to limit their presence being the lack of young developing roots or other tissue suitable for invasion. Since cysts containing active larvæ were first recorded on July 23rd, it is evident that first stage larvæ found in the plant tissue prior to that date, had originated from overwintered cysts. After July 23rd it was impossible to determine whether the first stage larvæ found in the tissue had escaped from overwintered cysts, or from cysts which had developed during the

current season. Under suitable conditions cysts continue to develop as late as October on stolons and tubers, and on tubers after stolons and fibrous roots have ceased to function. It is, therefore, possible for larvæ from the cysts of the current year to enter the root tissue and complete their development before the autumn.

Many females were still in the pyriform stage when the plants were destroyed by the autumn frosts. Although these appear sufficiently developed to assume the brown cyst appearance, they retain their pyriform shape, and on dissection are found to contain comparatively few eggs. Similar conditions follow the premature destruction of infested rootlets during the growing season. This seems to explain the occurrence of the pyriform or flask-shaped cysts with their contents in various stages of development, frequently noted during the winter when soil is being examined for cyst content.

Normally the eelworms appear to enter the plant tissue through the fine lateral rootlets, and the primary roots and first formed secondary roots seem to be the most favoured sites for eelworm development. Eelworms, however, may occur in other parts of the plant tissue, and the writer has found them in the base of the stems near the old sett, in the stolons and on the new tubers.

The Influence of Soil Temperature on the Development of *H. schachtii*.

According to Wilke (1925) temperature and moisture have an important influence on the development of eelworms. In order to study the effects of soil temperature on the development of *H. schachtii*, records were kept of the soil temperature of the experimental plots, at a depth of six inches below the surface. The temperature of the soil was read daily from April 25th to July 25th, and potatoes were planted at intervals from May 9th to June 22nd. Weekly dissections of the root tissue from plants on the various plots gave data with regard to the development of the eelworms. It is realised that intervals of a week between examinations permits a rather large margin of error, but the plots were a considerable distance from the laboratory and more frequent visits could not be arranged.

The soil temperature rose fairly steadily from April 25th to July 25th,

when recording ceased. Eelworm infestation appeared to begin early in June, and increased steadily from the middle of June until about the middle of August, after which a slight drop in the numbers of eelworms was apparent. The following table gives the numbers of eelworms per 5 mm. of primary root tissue on various dates on which examinations were made.

TABLE I.

Date of Examination.	Eelworms in 5 mm. lengths of root issue.	Average.
June 11th ...	1, 2, 2, 3, 3, 5, 1, 2, 1, 0, 1, 4, 0, 0, 1	1·7
June 18th ...	2, 10, 4, 11, 16, 11, 7, 4, 14, 3, 3, 7, 0, 3, 0, 1, 0, 0	6·0
June 25th ...	1, 2, 5, 25, 8, 7, 2, 7, 6, 7, 3, 21, 13, 2, 4, 3, 4, 3, 1, 2, 0	6·0
July 2nd	3, 9, 5, 7, 13, 15, 10, 10, 6, 14, 20, 18, 14, 17, 2, 7, 6, 10, 3, 7, 3, 0, 2, 2	8·4
July 9th	15, 4, 6, 23, 11, 10, 4, 12, 5, 12, 7, 6, 21, 14, 14, 8, 10, 11, 5, 1, 6, 4, 6, 10	9·3
July 16th ...	5, 6, 9, 4, 6, 5, 7, 15, 11, 14, 9, 2, 7, 3, 6, 11, 12, 16, 6, 11, 14, 19, 14, 9	9·2
July 23rd ...	5, 15, 9, 16, 17, 10, 12, 5, 17, 7, 6, 18, 19, 10, 14, 7, 9, 16, 13, 12, 12, 6, 13, 7	11·3
July 30th ...	14, 8, 8, 9, 10, 8, 11, 5, 16, 3, 2, 2, 9, 12, 13, 8, 15, 6, 6, 15, 23, 11, 11, 10	9·8
August 6th ...	13, 9, 24, 17, 13, 8, 9, 8, 7, 8, 6, 8, 10, 6, 5, 10, 14, 22, 15, 15, 12, 4, 9, 8	10·8
August 13th ...	22, 8, 15, 16, 13, 14, 14, 10, 6, 12, 8, 13, 14, 15, 21, 11, 16, 7, 15, 9, 10, 6, 6, 4	11·8
August 20th ...	9, 7, 7, 7, 3, 5, 4, 9, 7, 6, 6, 4, 16, 8, 7, 7, 8, 4, 9, 5, 5, 3, 2, 2	6·2
August 27th ...	5, 3, 6, 6, 10, 8, 2, 2, 1, 8, 14, 11	6·3
September 3rd ...	16, 6, 8, 8, 9, 10, 6, 8, 5, 10, 8, 5	8·2
September 10th ...	8, 5, 4, 3, 4, 4, 6, 7, 4, 5, 4, 3	4·7

The intensity of eelworm infestation appears to be influenced to some extent by the soil temperature. For the four weeks prior to the first records of eelworm intensity, the average temperature of the soil was 54° F., 59° F., 60° F., and 59° F. During the following week, June 11th to 18th, the soil temperature rose to 62° F., and eelworm intensity increased from 1·7 to 6·0 per 5 mm. of primary root. From these figures it would appear that there was some association between a soil temperature of 60° F. and the invasion of the root tissue by eelworms, but the clearness of this association is somewhat obscured by the fact that at this time roots were developing rapidly and the amount of tissue available for eelworm increasing correspondingly. From June 18th onwards to

August 13th eelworm intensity gradually increased, and appeared more or less parallel with the rising curve of soil temperature. Thus the general influence of soil temperature on eelworm activity, as indicated by Table I., appears to corroborate the conclusions of other investigators (Wilke, 1925) who state that the escape of larval eelworms from the cysts is directly influenced by temperature conditions.

Data was also obtained on the rate of development of the eelworms in the root tissue. This appeared to be practically uniform throughout the season. With potatoes planted between May 9th and June 22nd the time required until the protrusion of cysts from the root tissue varied between 47 and 38 days. Allowing a period of ten days for the development of secondary roots, the time required for the eelworms to reach the cyst stage appears to be about a month. In the case of potatoes planted in the first half of May cysts were not apparent until 47 days after planting, but this seemed accounted for by the fact that under the conditions of lower temperature prevailing in the early part of the season root development was less rapid and also the escape of eelworms from the overwintered cysts retarded. The period required for the development of eelworm larvæ within the cysts seemed to be about a month, and throughout the season larvæ were found in the cysts four weeks after cysts were first recorded on the plants on the respective plots. From these observations it seemed apparent that the life cycle of the eelworm on potatoes under north western conditions was completed in about two months, or a period of 64 to 75 days after the planting of the potatoes. Thus the life cycle was completed as early as July 23rd in the case of potatoes planted during May, and by September 3rd in the case of potatoes planted as late as June 22nd.

Influence of Soil Conditions on Potato Eelworm.

Some attention has been paid to the relationship of the potato eelworm and soil conditions, and the variants which have been considered are acidity as reflected by pH, and soil moisture and temperature as reflected by hygroscopic moisture and loss on ignition. With regard to the latter Smith (1929) concludes that there is no simple association, and that it appears improbable that the rate of reproduction of *H. schachtii* is influenced to any marked degree by the normal variations in the physical condition of the soil. With regard to the relationship between

H. schachtii and soil reaction, the conclusions are conflicting. Peters (1926) concluded from a series of measurements taken in one field that there was "an indubitable correlation between pH and cyst concentration," while in miscellaneous samples the reverse sometimes occurred. As a result of their most recent investigations Morgan and Peters (1929) state that the conclusions previously arrived at tend to be reversed. Smith (1929) found a significant negative correlation in the case of 53 peaty soils and no significant correlation in the case of 25 sandy soils. Kemner (1929) states that the composition and reaction of the soil seem to have but slight influence on the intensity of potato eelworm, and that heavy infestation occurs on soils of different pH value.

From observations made during the course of these investigations it would appear that the chief factor governing eelworm intensity was the vigour of the host plants. Thus the cyst concentration of the soil would tend to increase after a vigorous crop, and might even show some decrease after a weak unhealthy crop, irrespective of pH value save in so far as it influenced the health of the potato plants.

From a consideration of the life history of *H. schachtii* on the potato plant it would appear that the direct influence of soil reaction will be felt most strongly by the first stage larvæ during the period spent in the soil between the escape from the cyst and the invasion of the root tissue of the host plant. The reaction of the soil might, at this period, influence the survival rate of the eelworms, and this might ultimately affect the cyst concentration of the soil. On the other hand, however, the presence of a vigorous, healthy potato crop would tend to neutralise any decrease in the number of eelworms which might result from an unfavourable soil reaction:

EELWORM INFESTATION AND HOST PLANT REACTION.

Where growing conditions for the host plant were satisfactory, the only noticeable reaction to eelworm infestation appeared to be a copious development of lateral roots. The lateral roots which were invaded by eelworms retained their normal appearance, except for the occurrence of slightly brownish areas where numbers of first stage larvæ invaded the tissue in close proximity to each other. The characteristic increase in the numbers of lateral roots following invasion by eelworms, suggests

that there is some loss in efficiency associated with eelworm invasion, and to balance this other lateral roots are developed. In spite of this increased root production the toleration of the potato plant to eelworm attack seems to be of a very high order. This toleration has been indicated by other observers who have stated that apparently healthy plants can be found with a considerable eelworm population, and was corroborated by Smith and Prentice (1929) who found a higher cyst concentration occurring outside areas of " potato sickness," and Smith and Miles (1929) who noted that an increase in the cyst content of the soil occurred only after a crop which was not a total failure.

In order to form some idea of the extent of toleration of eelworm infestation, potato plants which appeared normally healthy were examined at various dates during the period from July 2nd to August 6th. The average concentrations of eelworms per 5 mm. length of root tissue in eight plants were 17, 15, 13, 14, 14, 14, 15, and 15 respectively, while single lengths showed as many as 23 eelworms of both sexes. In spite of the high eelworm concentration in the plant tissue no ill effects could be detected from the condition of the haulms. In another plant which appeared normally healthy, 28 cysts were counted on a single 5 mm. length of root.

Further observations on the condition of the haulm of potato plants in relation to the intensity of eelworm attack bore out the conclusion that the presence of eelworms in the plant tissue does not necessarily react unfavourably on the plant health. Plants grown on land which appeared " potato sick " the previous season were classified according to the condition of the haulm. The root tissue of 24 plants with " excellent " haulms were examined, and were found to have an average concentration of 10·6 eelworms per 5 mm. length of root tissue. Eleven plants with " good " haulms showed a similar eelworm concentration. Ten plants, of which the haulms were described as " fair," showed an eelworm concentration of only 7·7 per 5 mm. length of root. The yields of the plots from which these plants were obtained indicated that a high eelworm concentration had little adverse effect, for when the plants were lifted they produced a yield of approximately 8 tons per acre. Considering that the land had produced a potato crop two seasons in succession, this represents a fair average yield for the district and suggests that the yield

was not directly affected by a high eelworm concentration in the root tissue.

After examining carefully several hundreds of eelworm infested plants during the past three seasons the writer is of the opinion that the potato possesses a high degree of tolerance of the eelworm *Heterodera schachtii* and that the mere presence of eelworms in the root tissue is not sufficient to produce pathological conditions in the host plant.

Heterodera schachtii IN RELATION TO "POTATO SICKNESS."

Heterodera schachtii has been definitely associated with potato sickness for a number of years, but previous investigators have indicated that the cyst concentration of the soil does not always accurately reflect the intensity of pathological conditions in the potato plants. In order to investigate further the possible relationship between *H. schachtii* and "potato sickness," observations have been made on the development of the eelworms in the root tissue in relation to the health of the plant. These show that when the normal growth of the plant is not impaired by other factors, its toleration of the eelworm is very high, and an average of 14—15 eelworms per 5 mm. of primary root may occur without setting up pathological conditions, even though invasion by eelworms occurs within a fortnight of the setting of the seed tubers. The collection of evidence corroborative of this conclusion is difficult, because in the field, in every case of "potato sickness" which has come to the writer's notice during three years in Lancashire and Cheshire and previously in Lincolnshire, the issue has been complicated by the presence of other factors besides eelworms.

In 1929 an experiment was planned to demonstrate that a high cyst concentration in the soil and a consequent high eelworm population, did not set up a pathological condition in the host plant. Steam sterilised soil from a diseased area was used as a basis for the growth of potato plants and varying amounts of similar unsterilised soil which had been passed through a 2 mm. sieve were added to vary the eelworm concentration. The cyst concentration of the series was as follows :—

TABLE II.

Pot.	Soil mixture.	Total viable Cysts (approx.)	Cysts per lb. of soil (approx.)
1 ...	8 lb. sterilised	0	0
2 ...	6 lb. sterilised ... ... 2 lb. unsterilised	2,000	250
3 ...	4 lb. sterilised ... ... 4 lb. unsterilised	4,000	500
4 ...	2 lb. sterilised ... ... 6 lb. unsterilised	6,000	750
5 ...	8 lb. unsterilised	8,000	1,000
6 ...	8 lb. ordinary field soil (unsieved)	8,000	1,000

The experiment was set up in duplicate. The seed used was "Great Scot," grown in the same type of soil in 1928 and being used in 1929 on eelworm infested land. After setting the pots were embedded in the soil of a garden well removed from the eelworm infested areas, and the plants subsequently produced were kept under constant observation.

In the autumn the plants were carefully lifted and the root systems examined. The following table gives the cyst infestation per half-inch of root, ten counts being made on each plant.

TABLE III.

Pot.	Cyst concentration per 1 lb. soil.	Cysts per half-inch of root.	Average.
1 ...	0	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0
2 ...	250	2, 7, 4, 2, 5, 3, 5, 4, 2, 2	3·6
3 ...	500	10, 7, 4, 3, 5, 9, 9, 3, 7, 6... ...	6·3
4 ...	750	5, 8, 9, 3, 5, 6, 9, 5, 6, 6	6·2
5 ...	1,000	5, 7, 6, 9, 6, 8, 6, 7, 5, 7	6·6
6 ...	1,000	10, 7, 12, 8, 10, 8, 12, 8, 3, 6 ...	8·4
1a ...	0	0, 0, 3, 1, 2, 1,* 0, 0, 0, 0... ...	0·7
2a ...	250	2, 2, 3, 6, 6, 6, 5, 2, 2, 3	3·7
3a ...	500	2, 5, 5, 5, 3, 1, 11, 5, 7, 8... ...	5·2
4a ...	750	9, 3, 4, 5, 5, 5, 4, 6, 6, 3	5·0
5a ...	1,000	10, 8, 8, 11, 5, 6, 8, 10, 10, 8 ...	8·4
6a ...	1,000	9, 7, 7, 5, 7, 9, 6, 3, 3, 8	6·4

* All found on one root within a space of two inches—evidence of incomplete sterilisation of the soil. No other cysts occurred on plants grown in sterilised soil.

From these figures it is apparent that there is a correlation between the cyst concentration of the soil and the subsequent infestation of the plant by eelworms, and that the production of cysts on the plants varied directly with the original cyst concentration of the soil.

Notes were made on the growth of the plant during the season and it is interesting to examine these in relation to the subsequent cyst production. No differences in appearance were noted between the original and the duplicate series.

TABLE IV.

Pot.	Early growth.	Mid season.	Autumn.	Root development.	Yield.
1 A & B	Rather backward	Fair growth, dark foliage	Good vigorous growth	Fairly good, very copious fibres	Fair
2 A & B	Good	Vigorous plant, healthy dark green foliage	Good growth, stature good	Good, copious fibres	Good
3 A & B	Good	Good healthy growth, stature good	Good growth, stature fair	Good, fairly copious fibres	Very good
4 A & B	Good	Healthy and vigorous, stature good	Good growth, fair stature	Good, fairly copious fibres	Good
5 A & B	Good	Foliage pale, upright habit, stature fair	Foliage yellow, stature poor	Fair, primaries short	Good
6 A & B	Very good	Apparently healthy, foliage pale green	Foliage yellowing, stature fair	Fair, primaries short, fibres poor	Very good

From the data given in this table it is apparent that eelworm intensity had little effect on the yield. The early checking of plants 1 a and b, and the dark foliage and sustained growth of the plants in the mixtures containing sterilised soil, are due to the effects of sterilisation. The early cessation of growth and the yellowing of the foliage in 5 a and b and 6 a and b was normal, though in sharp contrast with the other plants. During the season there were no differences in the health of the plants which could be associated with the intensity of the eelworm infestation of the plant roots.

When the roots of the plants were carefully examined in the autumn it was found that the fungus *Colletotrichum tabificum* was present on all the plants grown in pots to which unsterilised soil had been added,

particularly on 6 a and b where the micro-sclerotia were plentiful on the dead and dying roots. Attacked roots are shown in plate III. The presence of this fungus, confirmed by Mr. Holmes Smith, somewhat obscured the conclusions which could be drawn from the experiment. The occurrence of the fungus, and, in certain cases, the absence of stimulating effects resulting from sterilization of the soil, seems sufficient to account for the marked differences in the autumnal appearance of the plants.

Potato Sickness and the Occurrence of Fungi.—Since Morgan first pointed out the occurrence of the fungus *Corticium solani* Kühn in association with *H. schachtii* in potato sick areas, all subsequent writers have recorded the presence of the two organisms in cases of potato sickness. This has rendered extremely difficult the fixing of responsibility for the setting up of pathological conditions in the host plant.

Mr. H. H. Stirrup, of the Midland Agricultural College, has studied *Corticium solani*, and appears* to have arrived at the conclusion that it is normally saprophytic, but under certain conditions may become weakly parasitic ; moreover, he seems to regard the fungus as part of the normal fungus flora of potato roots. The observations of the writer during the past three years support this view, for examination of a considerable number of crops over a wide area in Lancashire and Cheshire has in every case revealed the presence of some stage of the fungus on the roots, stolons, tubers or at the bases of the haulm. Edwards (1929) has grown potatoes in sterilised soil inoculated with *Corticium solani*, and states that after an initial check as a result of a bad attack of *Corticium solani* on the stems, the plant recovered and made normal growth. Cheal (1929) subsequently confirmed this.

Under normal field conditions the infestation of potato plants by *Corticium solani* is practically certain. Mr. Holmes Smith and the writer examined successive crops of potatoes planted on the same ground in 1928 and 1929, and obtained the data given in Table V.

Very different weather conditions occurred during the two seasons. In the early part of the 1928 season frosts, cold winds and rain were prevalent and growth was uneven, while in 1929 conditions were favourable for healthy plant growth and development was even. In spite of these differences in the growth of the plants at the time when injury by

* In litt.

Corticium solani is most apparent, infestation by the fungus seemed constant.

That infestation by *Corticium solani* has little direct influence on the yield was apparent when these plants were lifted. The yield in 1928, when " potato sickness " was very evident, was 3·96 tons per acre, and in 1929, when for all practical purposes the intensity of *Corticium solani* was the same, a yield of 8·81 tons per acre was obtained. Since 8·81 tons per acre is a more or less normal yield for the district it appears that the potato can tolerate an apparently serious infestation by *Corticium solani* in the early part of the season, and has sufficient power of recovery to enable it to produce a satisfactory yield at the end of the season.

TABLE V.

Year.	Number of plants.	Normally healthy plants.	Severe lesions by *Corticium solani*.
1928 ...	26	5	21
	25	11	14
	25	8	17
	11	5	6
	25	4	21
	25	9	17
1929 ...	27	8	19
	9	3	6
	20	9	11

Average percentage of infested plants in 1928 ... ... 69·5
" " " " 1929 ... ... 64·3

The plants from which the data in Table V. was obtained were grown on eelworm infested land. Prior to the setting of the crop in 1928 the cyst concentration averaged 16·2 per 10 c.c. of soil, and in 1929 before the crop was planted the cyst concentration had increased to 21·5 per 10 c.c. of soil. In both years, therefore, the plants were contending against two organisms. In 1928 the yield was poor, only 3·96 tons per acre being obtained; but in 1929, after a greatly increased cyst concentration and practically the same infestation by *Corticium solani*, the yield was 8·81 tons per acre. This seems to indicate that under certain conditions potato plants can tolerate invasion by eelworms and attack by *Corticium solani*, and yet produce a normal yield. On the plants set at intervals

between May 9th and June 22nd, it was noticed that lesions resulting from attack by *Corticium solani* appeared about 10 days after planting, and the complete girdling of the stem, known as " stem canker," occurred about three weeks later. These plants were also heavily infested with eelworms from about 10 days after planting. In spite of the presence of the two organisms, the plants appeared healthy and produced a normal yield of about 8 tons per acre at the end of the season.

Colletotrichum tabificum, usually regarded as saprophytic, was found in 1923, 1924 and 1925 in potato sick areas in Lincolnshire, and again in 1929 in Lancashire and Cheshire in the roots of some plants in potato sick soils, in association with *Heterodera schachtii* and *Corticium solani*. Under field conditions this fungus appears to hasten the dying off of the plant in the autumn by promoting the destruction of the roots, but no further symptoms of attack have been noted. Under conditions of healthy growth the organism may probably be disregarded, but when it occurs in potato sick soils it deserves consideration as a contributory factor.

Examination of cases of " potato sickness " in Lancashire and Cheshire during the last three years has shown that the root eelworm, *Heterodera schachtii*, and the fungus, *Corticium solani*, are invariably present. Occasionally the fungus *Colletotrichum tabificum* is also present. A study of the relationships of these organisms to the host plant, both separately and collectively, seemed likely to produce results which might lead to the discovery of a primary cause of " potato sickness."

The observations which are recorded and discussed in the foregoing paragraphs suggest that the eelworm, *Heterodera schachtii*, does not of itself set up pathological conditions in potato plants, and that under normal conditions the potato plant has a high degree of toleration for this organism.

The presence of the fungus *Corticium solani* results in a pathological condition in the host plant, whether the eelworm *Heterodera schachtii* is present or not. The diseased condition is temporary and, though the check to growth can be observed in the field, the plants gradually recover, make good growth and produce a normal yield of tubers. This power of recovery from attack by *Corticium solani* is also evident when considerable numbers of *H. schachtii* are present in the root tissue.

Observations have also shown that under field conditions normal crops can be obtained from land obviously "potato sick" the previous season. In four separate instances in South Lancashire in 1929, normal crops were obtained from land where the potato crop failed completely the previous season, in spite of a high concentration of cysts in the soil and severe infestation by *Corticium solani* early in the growing season. The plants in these areas made normal healthy growth, and showed no other sign of unsuitable conditions than the temporary check owing to attack by *C. solani*. In other parts of the same or adjoining fields, under apparently identical conditions, plants showed obvious signs of distress almost from the outset. They straggled through in the spring, remaining stationary after a few inches of growth, and then either struggled on or died, the patches having all the characteristics of "potato sickness."

This state of affairs suggests the occurrence of other factors which at the outset appear to depress the scale against the plants and result in the occurrence of typical "potato sick" areas. The early dying of the primary roots, and subsequently the secondaries, explains the differences between the appearance of the haulms on "sick" and normal patches. This condition of the roots, which does not appear to be associated with eelworm activity, calls for investigation. The destruction of the roots appears to take place progressively from the tip backwards, but the cause of the death of the tips in the first instance has not yet been discovered.

EXPERIMENTS ON SOIL TREATMENT.

Since October, 1924, numerous experiments to test the efficiency of various soil dressings have been carried out. Most of the experiments have had for their aim the destruction of the eelworm *H. schachtii*, though Strachan and Taylor (1926) have experimented with artificial manures in relation to "potato sickness." Morgan (1925) used a number of chemicals both in the field and in pot experiments. In the field improved conditions followed the use of potash, salt and naphthalene, and in pot experiments the use of carbon bisulphide and calcium cyanide effected some reduction in the numbers of cysts on the roots of the host plants. Roebuck (1928) found that in the second season after the application of naphthalene, treated plots showed considerable improvement over the untreated plots. Edwards (1929) obtained promising results by using crude

naphthalene (drained creosote salts), carbon bisulphide, calcium cyanide and calcium cyanamide as soil dressings. It is worthy of note that the three experimenters claim that some benefit followed the use of naphthalene, and in two cases calcium cyanide was found to give some relief.

Field Experiments in Lancashire, 1928.

In the spring of 1928 arrangements were made to lay down trials in a field where potato sickness had been severe the previous year. The grower had decided to keep this particular land free from potatoes for several years, but he was sufficiently interested to co-operate in the experiment. The substances used as soil treatments were flake naphthalene (grade 16) and calcium cyanide.

TABLE VI.

Plot.	Cyst concentration. per 10 c.c.	Treatment.	Yield.	Yield per acre.
1	34·4	Naphthalene	7 lb.	37·8 cwt.
2		Cyanide	10 ,,	54·0 ,,
3	27·6	Control	6 ,,	32·4 ,,
4		Naphthalene	7 ,,	37·8 ,,
5	28·6	Cyanide	9 ,,	48·6 ,,
6		Naphthalene	8 ,,	43·2 ,,
7	33·8	Cyanide	14 ,,	75·6 ,,
8		Control	5½ ,,	29·7 ,,
9	32·2	Cyanide	15 ,,	81·0 ,,
10		Naphthalene	8 ,,	43·2 ,,
11	37·0	Cyanide (10 cwts.)	16 ,,	86·4 ,,
12		Control	5½ ,,	29·7 ,,

A strip of ground, one yard wide, was divided into twelve plots ten feet long. Four plots were treated with naphthalene at the rate of 5 cwt. per acre; four with calcium cyanide at the rate of 5 cwt. per acre; one with calcium cyanide at 10 cwt. per acre, and three plots were left as untreated check plots. A length of 2 yards occurred between each plot and this was left untreated. The plots were of necessity arranged in a continuous series, and cyst counts were made for every two plots. The dressings were applied in the drills and lightly covered with soil. The potatoes were set immediately. The date of setting was May 21st, and the soil temperature at mid-day was 50° F.

On all plots growth began fairly evenly, but in the course of a month growth had practically ceased on the untreated plots. On the plots treated with naphthalene the plants showed indications of distress in the early part of the season but they gradually improved in appearance. Even the best plants, however, could not be considered normal. On the plots treated with cyanide growth was slow at first, but gradually accelerated until by mid-summer the plants showed a fair, but by no means good, haulm. This advantage was maintained until the plants were lifted on October 6th. By this date all the plants on the other plots had died down.

From the figures given in Table VI., the untreated plots yielded an average of 27·27 cwt. per acre. The plots treated with naphthalene at the rate of 5 cwt. per acre gave an average yield of 40·5 cwt. per acre. The plots treated with cyanide at the rate of 5 cwt. per acre produced an average yield of 64·8 cwt. per acre, while plot 11, which received cyanide at the rate of 10 cwt. per acre yielded at the rate of 86·4 cwt. per acre. Owing to the small dimensions of the plots the value of these figures cannot be insisted upon, but they appear to indicate that some benefit was derived from the application of naphthalene and calcium cyanide, and therefore confirm the results of other investigators.

Field Experiments in 1929.

Since observations made in 1928 indicated that some of the advantages derived from the use of calcium cyanide might be owing to nitrogen stimulation, and since the cost of this material would render its use on a large scale impracticable, it was thought advisable to test the effects of dressings of calcium cyanamide, particularly as the work of Edwards (1929) had shown that this material was of some value in reducing losses caused by " potato sickness."

Plots, 30 square yards in area, were laid out on " potato sick " land which has a cyst concentration of 26·8 cysts per 10 grams of soil. Dressings of calcium cyanamide, varying from 3/4 cwt. to 6 cwt. per acre, were applied and appropriate check plots were left untreated.

The plants on all the plots suffered from attack by *Corticium solani* in the early part of the season, and heavy infestation by *Heterodera schachtii* was general. There was a complete absence of symptoms of " potato sickness " in spite of the fact that it had been abundantly present in the area during 1928.

From the outset the plants on the treated plots made good growth and the amount of haulm seemed to be in direct relation to the amount of cyanamide applied. Plants on the untreated plots made fairly good growth, but they were paler in colour and the lower leaves turned yellow prematurely. In the autumn the plants on the untreated plots died down before those on the treated areas. The crop was lifted on October 7th, and at that time the plants which had received the heaviest dressings of cyanamide were still carrying large green haulms and had not fully matured. The yields from the plots are given below.

TABLE VII.

Plot.	Cyanamide per acre.	Yield per plot.	Average yield per acre.	
1	¾ cwt.	150 lb.		
1a	,,	108 ,,	9·59 tons	
2	1½ ,,	148 ,,		
2a	,,	106 ,,	9·14 ,,	
3	3 ,,	166 ,,		
3a	,,	133 ,,	10·47 ,,	
4	4½ ,,	176 ,,		
4a	,,	138 ,,	11·30 ,,	
5	6 ,,	153 ,,		
5a	,,	119 ,,	9·79 ,,	
6	Untreated	115 ,,		
6a	,,	98 ,,	7·67 tons	
7	,,	112 ,,		Average 7·90 tons
7a	,,	113 ,,	8·10 ,,	
8	,,	108 ,,		
8a	,,	106 ,,	7·70 ,,	

The average yield of 7·9 tons per acre from the untreated check plots is considered satisfactory, since this was the second crop of potatoes in two years. The healthy appearance of the plants throughout the growing season and the satisfactory nature of the yield afford further evidence that under certain conditions potato plants have a high degree of toleration for *H. schachtii* and can outgrow the effects of early attack by *C. solani.*

The general conclusion to be drawn from the figures in Table VII. seems to be that on " potato sick " land the application of calcium cyanamide increases the yield of potatoes. This suggests that, under conditions prevailing in the North West, the stimulation afforded by non-organic nitrogenous dressings may assist the plants to overcome

the effects of "potato sickness." The influence of extra nitrogen proves the more interesting in view of the fact that in experiments carried out under identical conditions in collaboration with the Ministry of Agriculture, no appreciable benefits accrued in 1929 from the use of crude naphthalene, flake naphthalene, and bleaching powder.

In conclusion it may be stated that where "potato sickness" has existed the previous season the growth of potato plants can be stimulated by the use of such chemicals as naphthalene and calcium cyanide, but the results produced are not always such as to justify the use of costly materials.

The application of extra nitrogen, in the form of calcium cyanamide, has in 1929 greatly increased the yields of potatoes on "potato sick" land. This would suggest that its use as a stimulant for potatoes in some areas of the North West is worthy of consideration.

ACKNOWLEDGMENTS.

The writer is indebted to Mr. E. Holmes Smith, Adviser in Mycology, Manchester University, for assistance in dealing with the mycological phases of the investigation, and to Professor R. T. Leiper for his helpful criticism of the MSS.

REFERENCES.

CHEAL, W. F., 1929.—" On the Cause of 'Potato Sickness,' " *Gardeners' Chronicle*, August 17th.

EDWARDS, E. E., 1929.—" The Control of a Serious Potato Trouble," *J. Minist. Agric.*, Vol. XXXVI., pp. 234-242. (*W.L.* 11365.)

KEMNER, N. A., 1929.—" Potatisnematoden eller potatisalen (*H. schachtii* subsp. *rostochiensis* Woll.)," *Medd. No. 355, fr. Centralanst. Lant. ent. Av.*, No. 56, 76 pp.

MORGAN, D. O., 1925.—" Investigations on Eelworm in Potatoes in South Lincolnshire," *Journ. Helm.*, Vol III., pp. 185-192.

— 1926.—" Some Remarks on the Etiology of Potato Disease in Lincolnshire," *Journ. Helm.*, Vol. IV., pp. 49-52.

Morgan, D. O., and Peters, B. G., 1929.—"The Potato Eelworm in Lincolnshire," *Journ. Helm.*, Vol. vii., pp. 63-80.

Peters, B. G., 1926.—"*Heterodera schachtii* (Schmidt) and Soil Acidity," *Journ. Helm.*, Vol. iv., pp. 87-114.

Roebuck, A., 1928.—"Potato Eelworm," *Fruitgrower*, January 12th, pp. 51-52. (*W.L.* 8745.)

Smith, A. M., 1929.—"Investigations on *H. schachtii* in Lancashire and Cheshire," Part II, *Ann. App. Biol.*, Vol. xvi., pp. 340-346. (*W.L.* 1025.)

Smith, A. M., and Miles, H. W., 1929.—"Investigations on *H. schachtii* in Lancashire and Cheshire," Part III., *Ann. App. Biol.*, Vol. xvi., pp. 596-601. (*W.L.* 1025.)

Smith, A. M., and Prentice, E. G., 1929.—"Investigations on *H. schachtii* in Lancashire and Cheshire," Part I., *Ann. App. Biol.*, Vol. xvi., pp. 324-339. (*W.L.* 1025.)

Strachan, J., and Taylor, T. H., 1926.—"Potato Eelworm," *J. Minist. Agric.*, Vol. xxxii., pp. 941-947. (*W.L.* 11365.)

Strubell, A., 1888.—"Untersuchungen uber den Bau und die Entwicklung des Rubennematoden *H. schachtii*," *Zoologica*, Stuttgart, Vol. ii. (*W.L.* 23820.)

Thorn, G., 1926.—"Control of Sugar-beet Nematode," *U.S. Dept. Agric. Farmers' Bull.*, 1514, 20 pp.

Wilke, S., 1925.—"Nematodes," *Handbuch der Pflanzenkrankheiten* (Sorauer), Bd. iv., pp. 3-55.

Plate I.

Typical plant from "potato sick" area, characterised by small haulm, loss of lower leaves and dearth of roots. (Nat. size.)

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp*. 103-122.

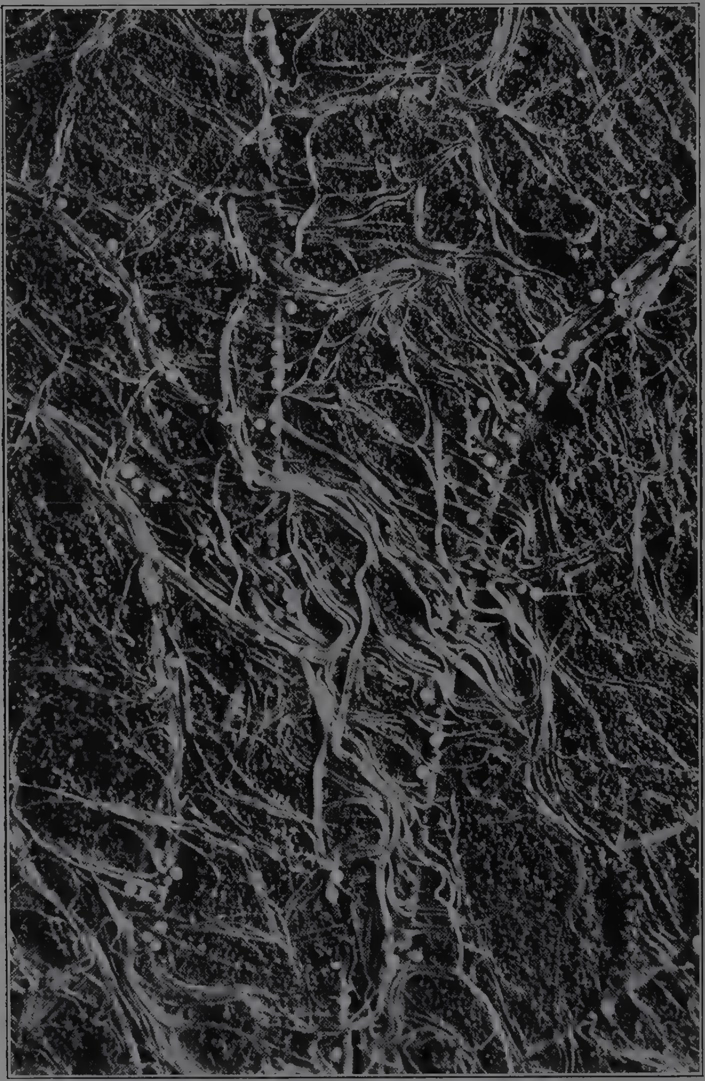


Plate II.

Roots of potato grown in "potato sick" soil; note lack of root hairs, eelworm cysts following the primary roots and comparative scarcity of lateral roots. (X 5.)

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp*. 103-122.

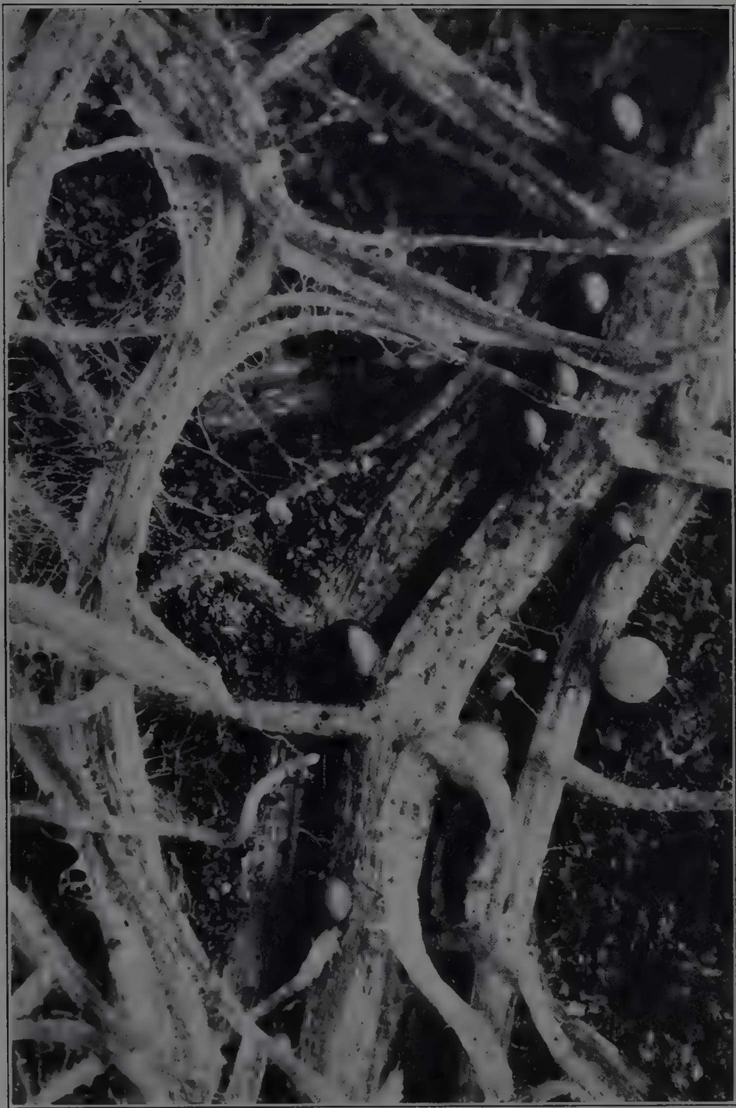


Plate III.

Potato roots showing eelworm cysts and small black micro-sclerotia of colletotrichum. (X 25.)

Journal of Helminthology, Vol. viii., *No.* 2, *June,* 1930, *pp.* 103-122.

Plate IV.

Clusters of white eelworm cysts on stolon; note brown mycelium of *Corticium solani*. (X 22.)

Journal of Helminthology, Vol. viii., *No.* 2, *June*, 1930, *pp*. 103-122.

Journal of Helminthology, Vol. VIII, *No.* 3, *September*, 1930, *pp.* 123-132.

On the Nematode Parasite of the frit-fly, *Oscinella frit*, L.

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

Introduction.

In a paper recently published in the Philosophical Transactions of the Royal Society of London, the writer describes the discovery of certain small Tylenchoid nematodes in the swollen stems of the seedling oats suffering from frit-fly attack at Winches Farm, St. Albans. Adults of both sexes and larvæ undergoing the last two moults were found in the destroyed plant tissues surrounding the fly larvæ. The spermatized female worms were next discovered within the body-cavity of the frit-fly larvæ. In due course, they were obtained from pupæ of the fly, having increased in size, and finally were found within adult flies of both sexes lying coiled within the abdomen as comparatively large sausage-shaped worms. As a result of the presence of the worm, the flies are sterilised, being unable to develop their reproductive organs. The worms become viviparous and shed large numbers of larvæ into the body-cavity of the fly and these, after undergoing a certain amount of growth, make their way into the gut of the host and pass to the exterior via the anus. In the present communication a brief account is given of the principal observations on the new parasite, its life-history and the effects on the host, described in detail in the original paper.

Morphology.

(a) *Adults, free-living.*

(i) *Male.* Length, 0·55 m. to 0·65 mm., width, 0·015 mm. to 0·02 mm., anus to tip of tail, 0·034 mm., anterior end to excretory pore, 0·12 mm., spicules, 0·011 mm., gubernaculum, 0·003 mm. Proportions, $\alpha = 36\text{-}37$, $\beta = 5$, $\gamma = 16\text{-}18$. As the above measurements

and proportions show the male is a small slender worm. The body tapers a little anteriorly whilst the head is rounded and is set off from the body by a slight constriction. The cuticle is finely striated transversely and there are distinct lateral fields with winged edges. There is no mouth or buccal stylet. The œsophagus is degenerate with a poorly defined outline and there is no intestinal gland such as occurs in the female.

The gonad is well developed and extends anteriorly in the body, tapering towards the fore end. It contains large numbers of extremely small round spermatozoa which occupy the bulk of the organ; only a small anterior region remaining as generative tissue. There is a pair of small spicules each having a rather broad head and tapering distally to a point. The gubernaculum is small and simple. On each side of the tail there is a narrow cuticular ala with a crenate edge very similar in appearance to that found on the males of many species of Tylenchus.

(ii) *Female.* Length, 0·5 mm. to 0·6 mm., width, 0·015 mm. to 0·02 mm., anterior end to end of œsophageal region, 0·125 mm. to 0·128 mm., anterior end to excretory pore, 0·117 mm. to 0·12 mm., anus to tip of tail, 0·03 mm. to 0·032 mm., vulva to tip of tail, 0·058 mm. to 0·06 mm., length of buccal stylet, 0·02 mm. to 0·021 mm. Proportions, $\alpha = 33\text{-}36$, $\beta = 4{\cdot}4\text{-}\ 4{\cdot}6$, $\gamma = 18\text{-}19$.

The body tapers a little anteriorly and posteriorly. The head is only faintly set off from the body. The tail is blunt and bears one or two small irregular processes on its ventral side. The mouth is very small and terminal. The buccal stylet is a comparatively massive structure, practically cylindrical in shape but with a short, steeply conical anterior region. Its walls are stout but thin down a little posteriorly where they join to the narrow lumen of the first part of the œsophagus. There are no basal thickenings of the walls of the stylet such as are found on the stylet of Tylenchus species. A lateral duct opens on the dorsal side into the lumen of the œsophagus at about 0·03 mm. from the base of the stylet and serves as the outlet of the intestinal gland. The latter extends from a short distance anterior to its duct backwards along the remainder of the œsophagus and about halfway down the intestine. Its hind end is rounded and the substance of the gland is made up of lightly refractive, finely granular spheres. It contains a single large nucleus.

The nerve-ring crosses the gland and the œsophagus at about the level of the excretory pore. The vulva is inconspicuous and lies a short distance anterior to the anus. It is connected with the uterus by a short, thick walled vagina almost at the end of the organ. The uterus at this stage is seen as a rather elongate tube about 0·12 mm. long, crowded with rounded spermatozoa. At its anterior end is a short length of cells, constituting the beginnings of the oviduct, and then a column of three or four large cells, each with a large nucleus, forming the ovarian primordium.

(b) *Parasitic female.*

Having entered a frit-fly larva, probably through the skin, the gonad of the worm begins to grow. The ovarian cells rapidly multiply and produce a large ovary which grows forwards in the body of the worm. At the same time, the oviducal cells multiply and the lengthening organ becomes folded on itself. A swelling makes its appearance at the posterior end of the oviduct and represents the incipient receptaculum seminis. At a little later stage of growth the spermatozoa pass into it from the uterus. The body of the worm now increases in length and width, apparently by the enlargement of the individual cells of the body-wall which become very vacuolate in appearance whilst their nuclei also enlarge. The stylet is retained throughout the parasitic life and the excretory pore and anus can be seen for a long time in growing worms. Ultimately the worm may attain a length of 1·6 mm. and a breadth of 0·13 mm. It is coiled and sausage-shaped by the time the adult flies emerge from the pupæ and lies free in the abdomen of male and female flies. From one to four have been found in a single fly and when more than one is present each is smaller than when one alone occurs. The ovary continues to grow forward in the body until it lies coiled on itself right up in the head end of the worm. By this time ova are beginning to detach themselves from the posterior end of the ovary. They pass down the oviduct, through the receptaculum seminis, where they are fertilised and into the uterus where they undergo segmentation and embryonation. The embryos hatch within the uterus and the parent worm thus becomes viviparous. The uterus at this time increases greatly in size and grows forward carrying the receptaculum seminis with it into the fore part of the worm.

Larvæ are shed into the abdominal cavity of the host and there undergo considerable growth accompanied by one or two moults. They finally make their way into the gut of the fly and pass to the exterior via the anus.

(c) *Larvæ.*

The larvæ as passed into the abdominal cavity of the host are very small, measuring 0·21 mm. to 0·23 mm. in length by 0·008 mm. to 0·01 mm. in width. The head is knob-like, is separated from the body by a slight constriction and the tail is rather bluntly rounded. There is a narrow buccal tube leading into a fusiform œsophagus which is about 0·03 mm. long. The intestine is made up of about 16 cells and is connected with the anus by a short rectum. The tail measures 0·02 mm. long.

Growth takes place until the larvæ reach a length of from 0·46 mm. to 0·5 mm., when they make their way into the host's intestine. Larvæ about 0·46 mm. have been observed undergoing an ecdysis, probably the second one. Male and female larvæ can be distinguished from the first on becoming free in the abdomen of the fly by reason of the different shape of the genital primordium and because of the presence in the female of the primordial cell of the intestinal gland.

(i) *Male.* In the male the genital primordium, which lies practically midway down the intestine on the ventral side, is rather elongated and measures about 0·04 mm. long by 0·005 mm. wide. It is composed of from 12-14 polygonal cells each with a large nucleus. At the anterior and posterior end there is a special terminal cell. The anterior one by its subsequent divisions, gives rise to the tunica propria covering the testis, whilst the posterior one ultimately produces an elongate group of cells from which the vas deferens is formed. By the multiplication and growth anteriorly and posteriorly of the large polygonal cells an elongated gonad is produced lying to the ventral side of the intestine. Spermatozoa are formed in large numbers and occupy the posterior half of the organ by the time the larvæ migrate into the gut of the host. Spicules are formed from two groups of spicule-mother cells which arise close to the anus. After passing out from the anus of the fly, the larvæ undergo two final moults in the free-living condition and the worms assume their adult organisation. The œsophagus loses its lumen and becomes degenerate in appearance.

(ii) *Female*. The female larva has a much smaller genital primordium than the male, composed of one or two large central cells with a terminal cell at either end. As in the male, the anterior terminal cell gives rise to the tunica propria whilst from the posterior terminal cell, the oviduct and uterus are finally formed. The whole primordium, with the elongating products of the posterior terminal cell, gradually moves backwards in the body of the larva and comes to lie a little in front of the anus. A plate of ectodermal cells now appears on the ventral body-wall and at its centre the cells become ridged and folded and ultimately give rise to the vagina and vulva. Finally a junction is effected between the double column of cells forming the uterus and the vaginal group. These last stages of growth are undergone as the female larvæ are passing through their two final moults in the free-living condition. At this time also the stylet is laid down.

In addition to possessing a smaller genital primordium than the male, the female larva, as already mentioned, is distinguished by the presence of a special elongated granular cell lying on the dorsal side of the intestine a little in advance of the genital primordium. This is the cell destined to become the intestinal gland. It contains a large nucleus and throughout the whole of the larval growth within the host's body, maintains its position on the dorsal side of the intestine, increasing a little in size as growth of the larva proceeds. During the two final moults it grows forwards in the body, along the dorsal side of the intestine and the œsophagus, and the duct connecting it to the lumen of the œsophagus, is laid down. In the male there is no sign of a corresponding unicellular gland at any time during development.

Effect on Host.

The presence of the parasitic female worm within the abdominal cavity of male and female flies results in their sterilisation. The external characters of parasitized flies are in no way different from normal ones; the only effect of the worm appears to be in checking the growth of the gonad proper.

(i) *Male*. The reproductive organs of a normal male frit-fly consist of a pair of brown pyriform testes about 0·4 mm. long by 0·2 mm.

in greatest width. From the base of each a vas deferens leads to a common ejaculatory duct whilst close to the insertion of the vasa deferentia there opens on either side of the ejaculatory duct a large cellular accessory gland.

In the parasitized male the testes remain very small and in a rudimentary undeveloped condition, whilst the accessory glands fail to develop. Each testis has a somewhat thickened wall and may contain a small group of undifferentiated cells, or the centre may be occupied with a reticulum of protoplasmic strands in which occur scattered nuclei.

(ii) *Female.* In the normal female fly there is a pair of pyriform ovaries varying in size according to the degree of development of the ova. The ovaries are connected basally by their oviducts with a common oviduct in a T or Y-shaped arrangement, and this leads posteriorly into the vagina. Arising from the latter are two long clear-walled ducts which gradually become narrower and much coiled. These are the ducts leading to the spermathecæ. Each ovary is made up of about 12 polytrophic ovarioles in each of which numerous ova gradually develop.

In the parasitized female the oviducts, common oviduct, vagina and spermathecæ and their ducts are as in the normal fly; the effect of the presence of the worm being shown in the undeveloped condition of the ovaries. These are small and rudimentary and although the outlines of the ovarioles can be distinguished within them, each ovariole is composed of a large number of a small undifferentiated cells.

One or two cases were observed of parasitized flies having normal well developed reproductive organs. In each case the worms in the abdominal cavity were small and showed signs of degeneration. The view is put forward that the parasite, during its development within the host, pours out some substance which checks the growth of the gonad and, *vice versa*, the gonad, if once it can develop, produces something which can prevent the full development of the parasite. In the great majority of cases the parasite succeeds in growing to maturity with sterilisation of the host, but occasionally, the reverse happens and the host comes successfully through the conflict.

Life-History.

The frit-fly, *Oscinella frit* Linn, has three generations during a year. Adults, of the generation which overwinters in the larval condition on wild grasses, emerge during May, being most abundant towards the end of the month. The females lay eggs on seedling oats which are particularly susceptible to attack up to the four-leaf stage of growth. The eggs hatch and the resulting larvæ tunnel into and destroy the central tissues of the stem. Attacked seedlings may be killed and as a rule are much stunted with thickened stems. The larvæ grow and finally pupate low down on the plant during the latter part of June. Adult flies of this, the stem generation, emerge during July, being particularly abundant round about July 17th. After pairing, eggs are laid on panicles of oats which are now appearing. A similar course of development is passed through; the larvæ destroy the soft green corn and finally flies of this panicle generation emerge during the latter part of August and early September. From the eggs of these, which are laid on various kinds of wild grasses, larvæ emerge which continue to feed on the plant throughout the autumn and winter months. Finally pupation takes place and the adult flies emerge in May as already stated.

Linked to the foregoing life-cycle of the host is that of its nematode parasite. Adults of both sexes and ensheathed larvæ, which have come from parasitized flies of the overwintering generation, are found in the free-living condition, in swollen oat stems containing frit-fly larvæ. The spermatized females then enter frit-fly larvæ and remain within the pupæ and adult flies. Here they undergo the growth changes already described and become viviparous. The larvæ, having grown and entered the gut of the host, pass out and are deposited by parasitized flies visiting panicles of oats in the course of their life-cycle rhythm. Making their way into the plant tissues surrounding frit-fly larvæ of the panicle generation, they undergo the two final moults and the spermatized females again become parasitic in the body-cavity of frit-fly larvæ. The parasitized flies emerging in August and September are sterilised and from their intestines the larvæ of the nematode again pass out. This time they are deposited on wild grasses and undergo the last two moults in the vicinity of frit-fly larvæ which have hatched from eggs laid by normal flies. Again the spermatized females enter frit-fly larvæ and remain within them during the winter months. Finally

when the adult flies emerge in May, the worms have developed and the larvæ are again passing out from the anus ready to continue the life-cycle in oat stems attacked by frit-fly larvæ of the stem generation.

Incidence of Infection.

(i) *Stem Generation.* Numbers of flies were collected from an experimental plot of oats at Winches Farm by sweeping with a white muslin net on July 17th and 20th. These were killed and subsequently dissected to determine the degree of infestation by the nematode.

No. dissected.	Females.	Males.	Infected.	Females.	Males.	% Infected.
966	607	359	142	68	74	14·7

These numbers show a large preponderance of females over males and yet, of those infected, there are more males than females. Flies of this generation were also obtained from Harpenden and the following numbers show the result of the dissection of these.

No. dissected.	Females.	Males.	Infected.	Females.	Males.	% Infected.
476	249	227	27	13	14	5·67

In this case the flies of each sex are practically equal in number and the infection is evenly distributed between them. At the same time the degree of infestation is considerably lower than in the flies from Winches Farm.

(ii) *Panicle Generation.* For this generation two small sheaves of oats were cut from the plot at Winches Farm and put into a breeding box on August 2nd. Flies were collected from August 8th to September 6th. The following numbers give the result of the dissection of these.

No. dissected.	Females.	Males.	Infected.	Females.	Males.	% Infected.
3,472	1,926	1,546	180	85	95	5·18

The degree of infestation for the whole period is much lower in this than in the stem generation of Winches Farm flies. A detailed table of dissections, given in the original paper, shows that the percentage infection is low in flies emerging during the first week but gradually rises to about 7 per cent. in those coming out during the period August 25th to 30th and remains at about this level to the end of the time of emergence. In this generation there is again a larger number of female

than male flies but the males show a higher degree of infestation than the females.

Geographical Distribution.

Flies of the panicle generation were received from Dr. A. D. Imms of Rothamsted Laboratory, Harpenden. These had been bred out from oats sent from different parts of the country. Dissection of these flies revealed the fact that the new nematode occurs at the following places :—Daresbury (Cheshire), Cambridge, Bridgewater (Somerset), Cardiff (Glamorganshire), Aberystwyth (Cardiganshire). Rugby (Warwickshire), Sandford (Oxfordshire), St. Albans and Harpenden (Hertfordshire).

Systematic Position.

In my original memoir the relationship of the new parasite to the following nematode genera, parasitic in insects, was examined and discussed, *Sphærularia*, *Atractonema*, *Allantonema*, *Bradynema* and *Howardula*, with the result that it was not placed in any of them. A comparison was also made with members of the genus *Tylenchus* reported from insects and it was shown to differ from these also. It was further compared with plant-parasitic and free-living members of the genus *Tylenchus* and was excluded from this genus on morphological grounds.

The new genus, *Tylenchinema*, was set up for its reception with the following generic diagnosis.

Tylenchinema. *Free-living* forms, small slender worms about 0·5 mm. long. Male without mouth or stylet and with degenerate œsophagus ; tail tapering behind anus and bearing caudal alæ ; spicules paired, gubernaculum simple and small. Testis single and anterior. Female with well-developed stylet, without basal thickenings, mainly cylindrical and having a short conical anterior region ; œsophagus without muscular bulb but with a narrow lumen in anterior region into which opens the duct of the large intestinal gland ; latter extending from median part of intestine forwards into œsophageal region. Vulva close to anus, vagina very short leading into tubular uterus serving temporarily as receptaculum seminis, oviduct and ovarian cells few in number. Fertilised female enters larva of host fly.

Parasitic Stage. A sausage-shaped form usually more or less coiled, having greatly developed ovary ; spermatozoa becoming localised in

special swelling at end of oviduct, the definitive receptaculum seminis; uterus becoming of great size and occupying bulk of body. Viviparous.

Genotype. *Tylenchinema oscinellæ* Goodey, 1930.

Parasitic in body-cavity of frit-fly, *Oscinella frit* L., on oats. Free-living forms in débris of stem or panicle of oats in vicinity of frit-fly larvæ.

Reference.

Goodey, T., 1930.—"On a Remarkable New Nematode, *Tylenchinema oscinellæ* gen. et sp.n., Parasitic in the Frit-fly, *Oscinella frit* L., attacking Oats." *Philos. Trans.* B., Vol. ccxviii, pp. 315-343, Pls. 22-26. (*W.L.* 16192.)

Journal of Helminthology, Vol. VIII, *No.* 3, *September*, 1930, *pp.* 133-164.

A Biological Investigation of Sewage.

By B. G. Peters, M.Sc., Ph.D.

(*Research Scholar in Sanitary Science of the Grocers' Company, 1927-29.*)
(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Introduction.

A biological study of the fauna of sewage-treatment plants was pursued by the writer for two years, from June 1927, thanks to the generosity of the Grocers' Company. The programme laid down included a qualitative and quantitative survey of the fauna and a collection of data on such physico-chemical factors as hydrogen ion concentration and oxygen requirement, with a view to seeking correlations between the two groups of phenomena.

A colorimeter was employed for measuring the physico-chemical factors, and the construction and operation of this has already been described in this journal (Vol. VII, No. 4, pp. 201-214). The present paper is in the nature of a report on the work as a whole. Considerable time was given to a study of the relevant literature, as a result of which the main outlines of sewage treatment in general are traced in the section on "Theoretical Considerations." There follows a section on the various techniques employed by the writer in his investigations, the results of the latter being discussed in a third and final section to which are appended some miscellaneous notes. Few tangible results emerge. The field is a wide one, and the relations between the organisms and their peculiar environment are extremely complex. This paper is nevertheless presented as a report on two years of work which has been, to the writer, exceptionally interesting.

Special attention has been paid to the nematodes of sewage, which form a numerous and much neglected section of the fauna. They are mentioned in due course in the following pages; but, rather than render this paper excessively unbalanced, a full account of them has been reserved for subsequent publication.

In the previous paper mentioned above, the writer expressed his indebtedness to the Grocers' Company, particularly their assessor, Sir John Rose Bradford, and to Professor R. T. Leiper, under whose supervision the scholarship was held. Here it is necessary to thank several other benefactors. Sir Andrew Balfour very kindly allowed the writer to use his reprint collection and subject index of works on hygiene, and also was good enough to introduce him to the Medical Officer of East Ham, Dr. W. Benton. The latter, and Dr. T. S. McIntosh, Medical Officer of Hendon, kindly arranged with the respective Borough Engineers for him to visit their sewage plants. To the Borough Engineers themselves he is grateful, and also to the foremen and workmen at the two plants who were always most courteous and obliging.

Theoretical Considerations.

An extensive literature has accumulated around the subject of sewage treatment and disposal and around the many related problems. Thanks to such publications as the Bulletin of Hygiene, the "Reports on the Progress of Applied Chemistry" (Society of Chemical Industry), the United States Public Health Engineering Abstracts and the "Summary of Current Literature" of the Water Pollution Research Board (Department of Scientific and Industrial Research), it is possible to trace the more important works in the literature; full bibliographies are also given in such monographs as Kershaw's "Sewage Purification and Disposal" and Martin's "The Activated Sludge Process." In the present instance works actually referred to in the paper are alone included in the bibliography at the end: further references may be discovered from the sources just noted.

THE NATURE OF SEWAGE.

Sewage consists of domestic wash-water, human excreta, and culinary waste, together with a certain amount of rain water. It may or may not contain street-washings and some or all of the storm water, according to the sewerage system locally in use. Thus it varies greatly in quantity and quality from place to place, and this local variation is further influenced by the presence or absence of industries discharging trade wastes, and by the general habits of the population. Moreover, in any one sanitary district there are fluctuations in amount and variations in quality from time to time. Thus there are usually a maximum during

the morning and a minimum during the night; Sunday's sewage is scanty and weak; Monday's is copious and soapy. If storm water is to any extent included, there may be a sudden storm at any time, diluting the mixture and greatly increasing its volume. At the outset, then, the sanitary engineer is faced by the problem of having to deal with a putrefactive, watery medium liable to extreme variations in quantity and quality: and his disposal system must therefore be flexible to a considerable degree.

GENERAL AIM OF SEWAGE TREATMENT.

Omitting from consideration the direct disposal of sewage without treatment, by such means as farming (irrigation) or delivery into fish ponds, the general purpose of a sewage disposal plant may be said to be the production of an effluent reasonably free from pathogenic organisms and with a reasonably low oxygen requirement. The importance of the latter factor will become apparent when it is remembered that the effluent is usually discharged into some stream or river, which will normally contain living organisms. An effluent with a high oxygen requirement will deplete the stream of its dissolved oxygen, with the result that the contained organisms may die and thus transform the stream itself into a sewer. The oxygen demand of crude sewage is due mainly to the presence of organic compounds, of the three main groups—proteins, carbohydrates and fats—in various stages of decomposition; it may be partly due in some instances to the presence of inorganic oxidizable substances in trade wastes, but in this paper the unexceptional can alone be considered. These organic substances are present partly in the form of suspended solids, partly as sols (so-called "Colloidal Solutions"), and partly as true solutions. While the distinction between these forms is less fundamental, chemically speaking, than was formerly supposed, it is yet of real importance to the sanitary engineer. For the coarser suspended solids will settle out without difficulty and so can be removed, and the dissolved substances lend themselves readily to oxidation; but the sols present a more intractable problem. In fact, one of the chief difficulties of sewage treatment is largely a problem in applied colloidal chemistry, complicated by the presence of micro-organisms living in the sewage. Assuming an unexceptionable effluent to have been produced, there remains the problem of disposing of the sludge—the solids deposited from the crude sewage.

PROCESSES IN USE.

In general, sewage treatment may be said to occur in two stages: (1) The removal of solids and the coarser suspended matter, and (2) Treatment of the supernatant liquid to precipitate the colloids and to oxidize the organic compounds. The end-products are thus a clear effluent which may be discharged into a stream, and a putrefactive sludge which may be either subjected to further treatment or buried.

(1) The crude sewage first passes through screens and then through a "Detritus" or Grit Tank. This is simply a greatly widened section of sewer in which the sewage is not retained, the heavier grit particles settling out by reason of the retarded velocity. The sewage now passes, almost always, to large Sedimentation Tanks in which the suspended matter settles out as a sludge. Chemical flocculants may or may not be added. This sludge may be periodically drawn off in a fresh condition for treatment or direct disposal elsewhere, or, in Sedimentation Tanks of the Two-storey type like the Imhoff Tank, it may be retained in the lower storey and allowed to "ripen" or "digest" under anærobic conditions. The latter process, and the anærobic digestion of the sludge in separate tanks, both result in the production of gases (methane, carbon dioxide and nitrogen, with sometimes traces of hydrogen, oxygen or hydrogen sulphide, according to the process), and a black substance, "humus," which is relatively inert and inoffensive. In the case of separate digestion tanks there is also an effluent which requires oxidation and therefore joins the settling tank liquor. The gases may be used for heating purposes, and the ripened sludge or humus may form the basis of a fertilizer.

(2) The sedimentation tank effluent, an opalescent foul liquid but free from settlable solids, is now usually treated for removal of organic substances, a process involving oxidation. (Rarely, the liquid is discharged directly into some body of water where this is large enough to receive it without harmful effects.)

In the earlier days, the obvious method of fine-sand filtration naturally suggested itself; the effluent was good, but the process was very slow, and the sand was quickly clogged. A little fearfully, experimenters tried coarser filtering material—with surprisingly good results. To-day, screened clinker or gravel varying around 1 inch in diameter is used. Obviously, then, this is no true filtration. It is, in fact, a complicated

process depending for its efficiency on the presence of a slimy film, surrounding the stones and containing living organisms, with which film the organic particles of the sewage come into contact. The filter beds may be fed from above by troughs, sprinkler-nozzles or rotating distributors (" sprinkling filter ") or, where sufficient hydrostatic head fails, the beds may be alternately flooded and drained from below (" contact beds "). In the former case the beds are ventilated from below, ensuring a good supply of oxygen, and are practically continuous in operation. They do require occasional resting periods, however. Apart from the intermittent presence of flocs of film loosened from the stones, the effluent from such beds is clear and has a low oxygen requirement. Passage through a final settling tank (" humus tank "), to remove the flocs, is usual; after which the effluent is discharged from the plant.

Another system now coming into use is that known as " Bio-aeration " or the " Activated Sludge " system. An early attempt (1891) was made to improve the action of filters by forcing air through them from below, with excellent results. The next step was the aeration of sewage in tanks, in the absence of any filtering medium: this also was found satisfactory, the solids readily settling out as a sludge. At about the same time experiments had shown that green algæ and other organisms greatly assisted in oxidizing sewage, by reason of the oxygen they produce during photosynthesis. Working from these results, Ardern and Lockett of Manchester made the discovery that oxidation and clearing were greatly hastened by *retaining* the settled sludge in contact with freshly added sewage. The sludge appeared to become " activated " in some way: it was found that it would settle out readily, that it was well oxidized and therefore inoffensive, that it drained fairly easily (a big problem in handling ordinary sewage sludge), and that it had an unusually high content of nitrogen. The essentials for the process were found to be a well " activated " sludge, an ample supply of oxygen, and an intimate contact between the sludge particles and the incoming fresh sewage. Blasts of air were at first used, both aerating the sewage and keeping it well mixed: latterly it has been found sufficient to keep the mixture agitated by mechanical means, usually by paddles propelling the sewage and sludge along shallow channels. Vanes may be fixed to the channel walls to induce spiral currents which facilitate the dis-

tribution of oxygen absorbed at the air surface. The total length of such a channel (winding back and forth to economize space) may be over half a mile. By connecting the two ends of the channel, and placing a weir near the inflow but " upstream " of it, the tank is rendered quite continuous in operation : as much liquid flows over the weir as enters the tank. This liquid passes into settling tanks from which the clear effluent is discharged ; most of the sludge is pumped back into the bio-aeration tank, a proportion (for it is continuously accumulating) is removed for disposal. With such a plant, even the initial sedimentation tanks are sometimes dispensed with.

If crude sewage is delivered into a river in sufficiently restricted quantities, purification will result by natural means. It is, perhaps, a sanitary advance on this to irrigate sewage without treatment in the soil, as is still done on small sewage farms. From this process it is, as has been outlined above, a long and complex evolution, through slow sand filters and sprinkling filters, to the activated sludge process. Yet the working of a modern activated sludge plant presents a close analogy to natural purification in a river. The two are, in fact, based on identical principles, but the former has the supreme advantage of being controllable.

The processes described in this section, sedimentation, sludge digestion, filtration and bio-aeration, are variously combined in a number of ways at different treatment plants. Sewage treatment is still in its experimental stages, and different local authorities adopt different systems in striking the balance between efficiency and cost. But whatever pattern of unit or combination of units, each unit more or less conforms to one of the main types sketched above.

It will now be convenient to consider some of the fundamental factors involved in these processes.

FUNDAMENTAL FACTORS.

Perhaps the chief impression produced by studying the literature of sewage treatment is that the successive stages in its development have been based on quite empirical knowledge, and that many of the discoveries made have been largely accidental. To-day, some of the processes are beginning to be understood, but there is still much to

be learned, and still room for wide divergence of opinion on many points.

For years there have been sharply conflicting views on the importance of living organisms in the various processes. One school has sought to explain the results in purely physico-chemical terms, while the other has insisted on the biological factors. The result of this opposition has been fortunate, in that a sound knowledge of the physico-chemical factors is being acquired—surely a necessary foundation; while, to-day, the biological factors are being increasingly recognized. When the position is fully considered, a complex and variable medium containing organic and inorganic substances in an unstable state (many of them in colloidal form), and an enormous population of microflora and microfauna living in this medium, then the complexity and obscurity of the numerous problems will be realized.

The process of artificial treatment, equally with the process of natural purification, involves the breaking down of highly complex organic substances. The proteins (essentially, chains of amino-acids) appear to be partly decomposed before they reach the plant, and are represented there chiefly by ammonia and urea; the undecomposed proteins make up a large percentage of the sludge. Here, hydrolysis with the aid of enzymes yields amino-acids which are readily attacked and decomposed by bacteria, in turn yielding ammonia (chiefly anaerobic) and carbon dioxide (chiefly aerobic bacteria, respectively). Of the residue, it is probable that alcohols and fatty acids are still further decomposed (Buswell). Heukelekian (1927) maintains that the available carbohydrates are largely decomposed by bacteria of the colon group, yielding a high acidity which restricts the increase of the bacteria. The end products are carbon dioxide and water. Cellulose is an important carbohydrate in sewage, and appears to be little affected in sludge digestion, the duration of the latter process being too short for cellulose fermentation. Information appears to be scanty on the question of the decomposition of fats in sewage.

The production of ammonia from proteins has been noted above. In sewage, as in the soil, this ammonia may be oxidized to nitrite and nitrate by the specific organisms concerned (*Nitrosomonas* and *Nitrobacter*), the reaction occurring in the filters and in the activated sludge process, after the oxygen demand of the substances present has been satisfied. For this reason the presence of nitrates in the effluent is

often taken as a measure of purification. This view might be sound did sewage treatment involve only catabolic processes. Actually, however, while complex substances are being degraded, their products are at the same time being re-synthesized into the living protoplasm of the contained organisms. Buswell (1928) has made this clear in his chapter on "Sludge Digestion." The dead organic matter of sewage, dissolved colloidal and solid, is disintegrated and then assimilated into the bodies of the organisms. These excrete simple compounds and eventually die and decompose. But this does not imply a return to the precise initial condition, for much of the original chemical energy has been expended and dissipated as heat in the life-processes of the organisms. This cycle of alternate decomposition and assimilation will continue (say, in a digestion tank) until only an inert humus remains, carbon dioxide and other gasses having been given off, and energy having been dissipated. The whole process may be likened to the behaviour of a pendulum set swinging and finally coming to rest. Buswell traces the same cycle of events in the zooglœal slime surrounding the stones of a filter, and in the suspended flocs of activated sludge. The humus here appears adjacent to the stone or at the centre of the floc as the case may be, where something approaching anærobic conditions may be said to obtain. To this production of less adherent humus next the stone he would attribute the "sloughing" of flocs from filters.

This generalized outline may suggest how the production of stabilized and inoffensive products (effluent, humus and gases) from unstable and putrescible sewage is usually accomplished. The case of activated sludge is rather different. Here the organic matter may be thought of as assimilated into the bodies of the organisms comprising the flocs. A good effluent is produced, but (assuming there to be no separate sludge digestion) in the place of humus and gases there is a sludge of living organisms. As might be expected in such a plant, there is less nitrate in the effluent and more combined nitrogen in the sludge. Workers at the Rothamsted Experimental Station have shown (1927) that this is the only sludge of real promise as a fertilizer, containing as it does when dry some 6 per cent. of nitrogen in an easily available form. They have shown that this is due to the assimilation of ammonia by bacteria and the assimilation of the bacteria by protozoa, the dead

bodies of which largely constitute dried sludge. The protozoa must not be allowed to increase sufficiently to reduce the bacterial population unduly.

In concluding this section, some important physico-chemical factors may be noted.

Mention has been made of the addition of flocculants to sedimentation tanks. Efficient precipitation appears to be chiefly dependent on hydrogen ion concentration and temperature. Wigley (1926) suggests that alkalies and alkaline salts are best for precipitation, acids and acid salts causing the sludge to float. J. A. Wilson (1924) dealing with sludge de-watering, found that when the reaction of sludge was reduced from pH. 7·4 to pH. 3·4 by the addition of sulphuric acid, it filtered at five times the usual rate. He found that the sludge particles carried a negative charge, which the acid served to neutralize. The sludge particles have a maximum size at the isoelectric point (zero charge), and are smaller the greater the charge, negative or positive. Moreover, at this point colloids have least affinity for water. Hence the adjustment of the reaction to the isoelectric point should facilitate both sludge de-watering and precipitation. At the isoelectric point heat is a great accelerator of coagulation. Combining pH-adjustment, addition of alum, and a temperature increase to 180° F., Wilson found the rate of filtration increased 40 times.

In the matter of anaerobic sludge digestion also, reaction and temperature are important. Rudolfs (1927), working at New Jersey, finds the optima to be pH. 7·3 to 7·6 and 80° F. respectively. The presence of air is here deleterious to the work of the liquifying bacteria, and, as in the case of activated sludge, an equilibrium between bacteria and protozoa must be maintained. Fair & Carlson (1927) give somewhat lower optima: pH. 6·8 during the initial stage of digestion and pH. 7·2 during the final stage. When thus controlled the period of digestion is reduced to one third, or even less.

In the oxidation processes, filtration and bio-aeration, the main consideration is of course an ample supply of air. Probably the chief advantage derived from resting a filter periodically lies in the fact that air gains access to all parts of the filter. During continuous running the filter is liable to become clogged, and " ponding " results. Ponding

is naturally less marked the larger the stones. On the other hand the efficiency of the filter increases with the total area of film exposed to the sewage, and for a given size of filter this area is greater the smaller the stones. Thus a balance has to be struck between these two factors: this lies somewhere around a diameter of 1 inch for the stones, depending on local conditions. The mechanism by which particles come into contact with the film appears to be largely an adsorption effect; as Buswell states (1928), "Colloidal and soluble substances which lower the surface tension of water concentrate at the jelly-sewage interface." Once adsorbed, the substances are quickly attacked and removed by bacteria and other organisms of the film, making room for further adsorption. Purvis (1926) finds that the oxidation of sewage occurs more rapidly in soft (distilled) than in hard waters. Hard water, however, appears to precipitate colloids more readily:—"This would increase the amount of sludge deposited during tank treatment, thus reducing the load on the subsequent treatment processes employed . . ." These two factors therefore seem largely to cancel out.

Few biological data have been given in this section, it being more convenient to consider them in the section on "Discussion of Results."

Technique.

The experimental work in the present investigation has included the collecting of various samples, chiefly from the treatment plants at Brent and at East Ham, followed by qualitative and quantitative survey of the animal population, and (in some instances) determinations of the hydrogen ion concentration and oxygen absorption of the samples. In this section it will be convenient to describe the main features of the treatment plants in question, the methods used in the surveys, and the technique of the chemical determinations, in so far as this last point has not already been covered in the previous publication (1929).

Sewage treatment plants.

At Brent the Hendon Council has two plants, similar in type but entirely distinct functionally, the two effluents discharging separately into the river Brent. At East Ham the East Ham Council also has two plants

but these are dissimilar in type, though they have in common an initial mechanical screening unit, and a final settling or " Humus " tank from which the single effluent is discharged into the Thames. Thus four plants may be distinguished and will be referred to as " Brent A and B " and " East Ham A and B " respectively. All four are essentially aerobic in action containing no anaerobic units such as the Imhoff, tank.

A description of the plant " Brent A " may now be quoted from an unpublished paper by the writer (1928). " The sewage passes first through metal screens, and then through a meter which records automatically the rate of flow and the total quantity passed. The sewer then widens out into a detritus tank : it is not held, but merely slowed down so that the heavier grit-particles can settle to the bottom. Two tanks are available, one being cleaned while the other is in use. An open drain now conveys the sewage to one of a number of large settling tanks ; here the sewage is retained until most of the solids have been precipitated as sludge. This sludge is periodically drawn off, spread on the land to dry, and subsequently ploughed in. The supernatant liquid, containing particles in fine suspension, colloidal particles and substances in true solution, is now allowed to flow by gravity along an open drain for a distance of one or two furlongs ; this process is conducive to the absorption of oxygen from the air. The sewage is now fed to banks of ' Percolators ' or trickling filters which constitute the main feature of the system. Each filter is rectangular in plan and section and is composed of graded clinker of 1 inch to 1½ inches diameter. Each is fed with sewage by a battery of six rotating ' Distributors,' the distributor consisting of four radial arms perforated along one side with small holes and being rotated horizontally by the gravity-fall of the sewage. Of the four banks of filters, three are working at a time while the fourth is rested (and cleaned if necessary) for one day. Each bank is floored with perforated tiles through which the purified sewage flows, to be collected into drains and conveyed to a humus tank, where any solids derived from the filters can settle out. Though unusual in systems of this type, there is at Brent a second set of filters receiving the supernatant liquid from the humus tank ; the effluent from these attains to such a high degree of purity that it is safely run direct into the river Brent without the usual final settling tank." The plant

" Brent B " is essentially similar, except that the second set of filters is omitted ; after a single filtration the sewage passes into a humus tank, the effluent from which is directly discharged.

At East Ham, plant " A " consists of a " Bio-aeration " or " Activated Sludge " tank built on similar lines to the original one at Sheffield, aeration being effected by mechanical agitation in contradistinction to air-blowing. After mechanical screening the sewage passes into one of two detritus tanks from which it is fed to the aeration tank. The latter is divided by longitudinal partitions, incomplete at alternately opposite ends, so as to form one long narrow channel doubling on itself fourteen times. The sewage is delivered into the second channel and is propelled round its course by a set of paddles situated half-way down the tank. Adjacent paddles must move in opposite directions, of course, so that two driving shafts are necessary. On leaving the 14th channel the sewage is returned by way of a transverse duct to the first channel, the action of the tank thus being continuous. The outer wall of this transverse duct functions as a weir over which as much sewage leaves the tank as enters it at the opposite end. This excess sewage now passes into Dortmund settling tanks from which the clear effluent flows through a Lea Recorder to the humus tanks, while the settled sludge is pumped back to the aeration tank so as to maintain there the correct proportion of activated sludge to raw sewage (about 20 per cent.). Excess sludge—for it is continuously accumulating—is spread on the land to dry.

Plant " B " at East Ham consists of four chemical precipitation tanks, in which lime and iron sulphate are used to throw down the solids. The supernatant liquor is next sprayed from fixed nozzles over a large bed of clinker through which it is filtered. The filtrate now passes to the large humus tanks where it is joined by the effluent of the activated sludge plant. This humus tank is divided by walls into four compartments, the liquor passing from one to the next over a weir near the top of each wall. From the fourth compartment the final effluent is conveyed to the river Thames.

COLLECTION AND EXAMINATION OF SAMPLES.

From all four plants samples were collected in wide-mouthed jars and tubes for biological examination, and in narrow-necked bottles,

coated internally with paraffin wax, for chemical determinations. Apart from the various liquid samples, specimens of clinker from the filters at Brent and East Ham were also collected. For the less accessible units a long collecting stick was necessary. Samples were taken principally at the following points :—

Brent.
- Plant " A."
    - *1st Filters.*—Influent.
        - Clinker.
        - Effluent.
    - *Intermediate Humus Tanks.*
    - *2nd Filters.*—Influent.
        - Clinker.
    - *Final Effluent.*
- Plant " B."
    - *Filters.*—Influent.
        - Clinker.
        - Effluent.
    - *Humus Tanks.*
    - *Final Effluent.*
- River Brent.
    - Above effluents " A " and " B."
    - Below effluents " A " and " B."

East Ham.
- Plant " A."
    - Detritus tank.
    - Aeration tank—1st channel (purified sewage returned).
        - 2nd channel (raw sewage added).
    - Dortmund tank effluent.
    - Activated sludge (returned to aeration tank).
- Plant " B."
    - Chemical precipitation tanks.
    - Clinker from filters.
- Humus tanks.
- Final effluent.

Initial chemical determinations were made on the day the samples were collected; biological investigations not until the next day. Biological samples were transferred to Petri dishes and were self-cultured at room temperature (20° C.) with distilled water added to compensate for evaporation, or were cultured in tap water or in hay infusions. These cultural methods revealed a succession of organisms, some of which might have escaped notice in an examination of the fresh sample.

Organisms were observed in the living condition as far as possible, but for camera lucida drawings it was usually necessary for them to be killed and fixed in various ways, according to their nature. Thus, protozoa were fixed with osmic acid vapour or with Schaudin's fluid, oligochætes with hot formol-saline. Nematodes were killed by heat and examined without further fixation, this being the best way to avoid shrinkage and distortion.

For rapidly determining the dimensions of organisms without recourse to the camera lucida the following method was occasionally used. A graticule was inserted in the eyepiece of the microscope and rough drawings were made on paper ruled in ¼-inch squares, the draw-tube being so adjusted that, with a given objective and eyepiece, the side of a single square on the graticule corresponded to a convenient whole number of microns. Thus with a Leitz No. 3 objective, a Gifford 6 mm. eyepiece and a tube length of 140 mm., the sides of the graticule squares represented 25μ.

QUALITATIVE AND QUANTITATIVE SURVEYS.

These surveys entailed the rapid examination of large numbers of samples and, naturally, in the case of certain organisms immediate identification was impossible. To circumvent this difficulty a serial number was given in every case to the notes and drawings pertaining to each recognizable species, so that the more involved systematic work could be done subsequent to the routine examinations.

The central problem under investigation was the possibility of some correlation between the numbers and kinds of animal organisms present on the one hand and various physico-chemical factors on the other. For this purpose it seemed unnecessary to determine the actual species in every case, or to include rare transitory forms that might occur in

small numbers. The essential point seemed to be the accurate recording of certain of the more common forms, whether these were constantly present or appeared only sporadically in large numbers. A reliable record of selected key organisms rather than an exhaustive survey was the aim.

The selected animal organisms, each of which was identified at least as far as generic rank, totalled 52 and were distributed in the various groups as follows :—35 protozoa, 5 nematodes, 4 rotifers, 1 gastrotrich, 2 oligochætes, 2 crustaceans, 2 insects and 1 tardigrade. Their names will be found in the subjoined list.

List of Key-Organisms Selected for Observation.

Protozoa.

- Acineta fluviatilis.
- Actinophrys sol.
- Amœba proteus.
- Amœba radiosa.
- Anthophysa sp.
- Arcella vulgaris.
- Aspidisca costata.
- Cænomorpha medusula.
- Centropyxis aculeata.
- Cercobodo sp.
- Chænia sp.
- Chilodon sp.
- Chlamydomonas sp.
- Colpidium sp.
- Cyclidium sp.
- Difflugia sp.
- Euglypha alveolata.
- Euplotes sp.
- Holophrya sp.
- Lionotus sp.
- Mastigamœba sp.
- Microgromia sp.
- Notosolenus sp.
- Opercularia berberina.
- Paramœcium caudatum.
- Podophrya sp.
- Spirostomum ambiguum.
- Stentor cœruleus.
- Stentor polymorphus.
- Stylobryon sp.
- Stylonichia sp.
- Uronema sp.
- Vahlkampfia albida.
- Vorticella sp.
- Zoethamnium sp.

Nematoda.

- Diplogaster striatus.
- Diplogaster sp.
- Diploscapter coronata.
- Dorylaimus sp.
- Rhabditis sp.

Rotatoria and Gastrotricha.

- Chætonotus sp.
- Colurus sp.
- Diglena sp.
- Monostyla sp.
- Philodina sp.

Oligochæta.

- Æolosoma sp.
- Limnodrilus sp.

Crustacea.

- Asellus sp.
- Cyclops sp.

Insecta and Tardigrada.

- Achorutes viaticus.
- Macrobiotus sp.
- Psychoda sp.

Thirty-three other animal organisms, bacterial forms, fungi and algæ, identified or not, were also noted from time to time.

The various organisms were listed in a numerical register and also card-indexed, alphabetically under genus if identified and numerically if not. The cards gave the serial number, main systematic classification, references to drawings and notes and to relevant literature, and the ecological classification given in Whipple (1927, pp. 540-557). The latter is a sanitary ecology of aquatic forms modified from the system of Kolkwitz & Marsson (1909), and classifies under four zones: the polysaprobic, mesosaprobic (subdivided into alpha and beta zones), oligosaprobic and katarobic. It was thus possible to compare the forms in the various units of sewage plants with the ecological status previously given them by the authors quoted in Whipple's list.

The census of organisms was also compared with that determined by various workers at the sewage substation established by the Agricultural Experiment Stations and the State Department of Health at New Jersey.

In the quantitative estimation of organisms a home-made Sedgwick-Rafter counting cell was used. The cell, mounted on a glass slip, should have the following internal dimensions: length, 50 mm.; width, 20 mm.; depth, 1 mm. (capacity, 1 cc.). In the original a brass rim is used; in the present case glass strips were cut from a slip 1 mm. thick and cemented on with Canada balsam. The thickness of the slip (and the final depth of the cell) was estimated by means of the fine adjustment of the microscope, which had a vernier attached. The device was calibrated as follows. The thickness of a slip was determined by supporting it on edge on the stage and measuring with an eyepiece micrometer. The slip was then laid flat and the microscope focussed, by the fine adjustment, successively on to inked lines drawn at right angles on the two surfaces respectively. A correction for the refractive index of glass was then applied. The cell when completed had an actual capacity of 1·04 cc. To fill the cell a coverglass is placed on it askew, so that two diagonally opposite corners are open. The liquid is run in from a pipette at one corner and air escapes at the other. When the cell is full the coverglass is drawn into place automatically by surface tension. For relatively large organisms, the total number in the cell is counted; for small ones, a square eyepiece-stop is used and several

random fields counted. By adjusting the draw-tube and selecting a suitable stop, a convenient fraction of 1 cc. can be included in each field.

This method is most difficult to apply in the case of the film surrounding the clinker from the sprinkling filters. Probably the best way is to loosen and disintegrate the film by agitation in a known volume of water and then to determine the dry weight of solids from an aliquot.

PHYSICO-CHEMICAL FACTORS.

The factors selected for correlation with the biological data were hydrogen ion concentration and oxygen requirement. Determinations of both of these factors were originally made roughly in the field. As regards hydrogen ion concentration, indicator solution was added to sewage in a test-tube in the proportion of 1 drop to 1 cc., and the resulting virage was matched against the colour chart given in Clark's "The Determination of Hydrogen Ions." As regards oxygen requirement, the rough test was used which was suggested by Kershaw (1925) for effluents. To 25 cc. of sewage effluent are added 10 cc. of 10 per cent. sulphuric acid and 1 cc. of N/80 potassium permanganate. If the mixture is not decolourized in 3 minutes the effluent may be regarded as good. If 2 cc. are decolourized in the same time the effluent is poor or bad.

Subsequently a laboratory technique was elaborated, giving much more accurate results. The determination of both factors is amenable to colorimetric methods, and a colorimeter was constructed and calibrated giving readings for hydrogen ion concentration in pH.-values (to about pH. 0·05) and for oxygen requirement in milligrammes of oxygen required per litre, or parts per million (to about 0·2 mgm. per litre, or 0·2 p.p.m.). A full account of the construction and use of this colorimeter has already appeared in this Journal (Vol. VII, No. 4, pp. 201-214). The colorimeter was also used to measure the decrease in turbidity of samples centrifuged or allowed to settle.

As a routine test the absorption of oxygen was allowed to proceed for 10 minutes, readings being taken at intervals of 1½, 3, 6, 8 and 10 minutes. For the samples dealt with in this investigation the 10-minute oxygen requirement forms a useful standard for comparison. Special longer period tests were made in certain cases, up to 100 minutes, and other tests were made to find the result of storing the samples for several

days ; some curious results were obtained, but they do not concern the present problem. One important question was whether the sample should be filtered, shaken up, or allowed to settle for a definite time, before making an estimation. Typical results of the three treatments on a single sample of sewage are illustrated in the accompanying graph (fig. 1). Filtration undeniably gives the most manageable results, but

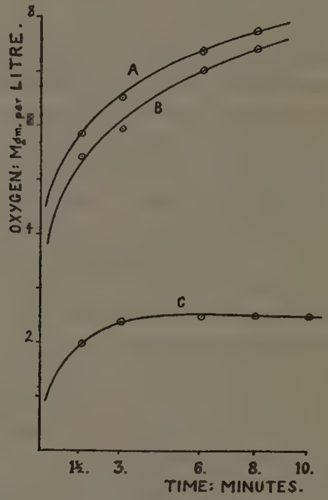


Fig. 1.—Curves showing the Oxygen Absorption of a sample under different treatments :—A, Shaken ; B, Settled ; and C, Filtered.

in the case of a settling tank or an effluent flowing into a stationary body of water (as the river Brent flows into the Welsh Harp) the sample settled for a definite time is nearer the natural condition. Moreover, an aerobic organism living in such a medium decidedly has to compete, with filterable particles for the available supply of oxygen. Accordingly determinations were made on settled samples as a matter of routine, and this is to be understood in the case of all results given below, unless otherwise stated.

Several seconds are required to take a reading, particularly when turbid solutions (necessitating the use of the comparator) are being

tested. For this reason there is a considerable time-error involved in the reading for $1\frac{1}{2}$ minutes. Moreover, it is difficult to match the virages accurately when most of the $KMnO_4$ has been reduced and the colours are weak. For this reason an increasing colour-error is involved for values above about 6·0 mgm. oxygen absorbed per litre (when the maximum capacity of the apparatus is fixed at 8·0 mgm.; for strong sewages the maximum is raised to 16·0 mgm., 24 mgm. or even 32 mgm., by adding more $KMnO_4$ to the samples).

Since temperature is a factor of some importance in these physico-chemical processes, the laboratory was maintained at a temperature very close to 20° C., by means of regulating windows, doors and radiator valves.

Discussion of Results.

The information to be gleaned from the masses of data recorded is, in many respects, disappointingly slight. Accordingly, to tabulate all the data here would be useless. Certain interesting facts emerge, as a result of drawing comparisons between various plants or units at different times, and these may be worth noting and illustrating by means of selected data. For this purpose it will be inconvenient to treat separately of the various factors; the latter may therefore be given some general consideration at this point. They are, principally, population of animal organisms, hydrogen ion concentration and oxygen requirement.

GENERAL RESULTS.

Variations in the population of animal organisms from time to time in a given unit are considerable; less so from one unit to another in the same plant, since the organisms tend to be carried passively from one unit to the next. The variations involve both absolute numbers of organisms and relative numbers of different species. To a large extent these variations seem quite random, species appear in considerable numbers for a time and then disappear again for no obvious reason. Nevertheless there is a general law governing the degree of variation met with, a law illustrated in the ecological classification of Kolkwitz & Marsson referred to above (p. 148). Briefly, in a highly polluted medium (polysaprobic zone) there are very large numbers of organisms belonging to relatively few species at any one time, different species rising to dominance and disappearing again with catastrophic violence.

This zone is characterized by the absence of dissolved oxygen, and the dominance of certain anaerobic bacteria and protozoa. In a less polluted medium (mesosaprobic zone) there are more species present, and changes in dominance are slower and less marked. Oxidation processes occur, and algæ, worms and rotifers appear. The oligosaprobic zone, in which mineralization is complete and the water is saturated with oxygen, and the katarobic zone (*e.g.*, pure spring water) with its few specialized species, have no place in a sewage plant. These ecological principles will be illustrated in special cases to be mentioned below.

The variations in hydrogen ion concentration, serially throughout a given plant and seasonally, are slight and, such as they are, not obviously related to any other observed factor. From these restricted data there is no indication of any of the organisms being controlled by a pH.-factor. Such control is illustrated by the work of Saunders (1924) who found that *Spirostomum ambiguum* would live only between the limits of pH. 6·0 and pH. 7·6, the optimum being pH. 7·4. This ciliate has occurred in the filters at Brent, at pH. 7·5, but there was an inadequate range of pH.-data to substantiate Saunders' work. Furthermore, there is no evidence of any relationship between the oxygen requirement and pH-value of sewage under treatment.

The variations in oxygen requirement are, naturally, very considerable serially throughout a plant, and are interesting as between one plant and another (as will be seen below); but they are not significant seasonally.

BRENT.

In the plant "Brent A," it may be recalled, there are two sets of filters in series. The clinker on the surface of these has formed the chief source of the biological material, while their influents and effluents have supplied samples for the physico-chemical determinations. The plant "Brent B" is essentially similar, with the omission of the second set of filters.

Physico-chemically considering the process as a whole, the filters receive a milky liquid containing a heavy suspension of fine solids and having a characteristic unpleasant smell. This liquid will take up 20 to 30 milligrammes of oxygen per litre in ten minutes from a solution of $KMnO_4$, and has a pH-value varying round about pH. 7·5 from pH. 6·5 to pH. 8·0. There is a large population of bacteria but few animal

forms—mainly small flagellates. The final effluent, on the other hand, is a clear liquid with very few suspended solids (said to be less than 3 parts per 100,000) and a slight earthy odour which is not at all unpleasant. The 10-minute oxygen demand is reduced to about 4 mgm. per litre. The pH-value may differ slightly from that of the influent; usually it is a little lower. There are comparatively few bacteria and few animal organisms, except at certain times when the film loosens from the clinker and comes away in flocs. These changes in the turbidity, oxygen demand and bacterial population are brought about while the sewage flows round the stones of the filters and comes into intimate contact with the slimy zooglœal film clinging to them, as has been described above in the section "Fundamental Factors."

A representative set of physico-chemical values for "Brent A" are shown in column 1 of Table I, while column 3 shows a set for "Brent B." It will be noted that in the latter case the effluent is not so good. This is because the influent is usually slightly stronger, and there is only the one set of filters. Moreover the values in column 1 are for July, and those in column 3 for November. In winter there is a tendency for the filters to become clogged and to "pond" at the surface, thus lowering the efficiency of the unit. This was particularly noticeable in the second series of filters at "Brent A," probably owing to the smaller-sized material used in them. Column 2 in the same table contains a curious anomaly which illustrates this point. The time is November, and a strong sewage has been particularly well oxidised by the first set of filters, the oxygen demand being reduced from 30 to 3 mgm. per litre. But part of this effluent is now fed to one of the second set of filters which is ponding badly. The result is that the effluent from this filter is actually inferior to that from the first set, the 10-minute oxygen demand having increased to 5·4 mgm. per litre.

Filters: First Set.

The organisms living in the "Brent B" filters and in the first set at "Brent A" are essentially similar. In the ecological system of Kolkwitz and Marsson they would be placed just within the meso-saprobic zone, bordering on the polysaprobic. There are large numbers of protozoa, especially small flagellates and ciliates, and nematodes, rotifers and oligochætes are also common. Of the 53 key-organisms

listed on page 147, 25 have been found altogether in this location. In addition, bacteria of the vibrion type are very commonly seen ; chain algæ or any other chlorophyllaceous organism only rarely and sparsely. The sporadic dominance of certain forms, characteristic of a saprobic medium, has been typified on various occasions. Thus in July, 1928, in one of the first filters at " Brent A," and in November, 1928, in the

Location.	Brent A.				Brent B.	
	Column 1, 24/7/28.		Column 2, 6/11/28.		Column 3, 6/11/28.	
	pH.	Oxygen.	pH.	Oxygen.	pH.	Oxygen.
Influent, 1st Filters	7·5	24·0	7·9	30·0	6·8	30·0
Influent, 2nd Filters	7·6	4·6	7·55	3·0	No 2nd Filters at Brent B.	
Effluent, 2nd Filters	7·45	3·2	7·45	5·4	6·95	6·0

Table I.—Physico-chemical data of Brent Sewages. For explanation, see text.

filters of " Brent B," the surface of the filter was swarming with a species of *Limnodrilus*. The habits of these oligochætes have been carefully studied by Purdy (1926), who finds that they prefer a highly polluted medium, and that they perform a useful service (at least, in polluted rivers) by ingesting subsurface mud and depositing it as small fæcal pellets on the surface. In the filters their principal contribution would seem to be dislodging the film from the stones. Again, in November, 1928, the surfaces of filters in the first set at " Brent A " were literally crawling with the larvæ, pupæ and imagos of the psychoda fly. This fly can be a great nuisance, particularly to people living near an infested sewage plant, and efforts have been made in the U.S.A. to control it by means of chlorination (see Cohn, 1925, and Enslow, 1927). At Brent, however, its appearance in any numbers seems to be quite sporadic.

Among the relatively larger organisms, the rotifer *Philodina* sp. was constantly present, and occasionally increased in numbers to a

position of dominance. Nematodes were also very numerous, but varied considerably from time to time both in total numbers and in particular species present. Thus, in a sample taken in January, 1928, *Diploscapter coronata* was the dominant organism of the film, yet it had not been seen before and apparently it disappeared shortly afterwards for it has not been found since. *Dorylaimus* sp., a very large form as free-living nematodes go, appeared plentifully in the first set of filters in the summer of 1928; by November it was rare in this location but common in the second set of filters. Various species of *Diplogaster* and *Rhabditis* were to be found at all times, and often in considerable numbers.

The seven following key-organisms, among the total of 25 found in the first filters at "Brent A" and in the "Brent B" filters, were not recorded from the second filters at "Brent A": *Acineta fluviatilis*, *Chlamydomonas* sp., *Stylobryon* sp., *Zœthamnium* sp., *Diploscapter coronata*, *Rhabditis* sp., and *Chætonotus* sp.

Filters: Second Set.

On passing to the filters of the second set, the change in the biological picture is most apparent. The vibrion-like bacteria are scanty, and the surface of the filter is often green with *Spirogyra* sp. and other filamentous algæ. While the total of organisms (especially if expressed as Standard Units) is less, the number of species present is considerably greater. Thus, of the key-organisms selected, in addition to the 18 species shared with the first filters, there were found to be 21 additional species in the second filters, making a total of 39, or a net increase of 14. Included in the 21 additional forms were such ciliates as *Spirostomum ambiguum*, *Cœnomorpha medusula*, *Stentor cœruleus* and *Lionotus* sp.; *Colurus* sp. and *Diglena* sp. among the rotifers; the primitive oligochæte (or archiannelid) *Aeolosoma* sp., and a tardigrade—probably *Macrobiotus* sp. But most characteristic of the fauna, and present in large numbers, were certain of the armoured rhizopods, notably *Arcella vulgaris*, *Centropyxis aculeata*, *Euglypha alveolata*, *Difflugia* sp. and *Trinema* sp. Of these, *Arcella vulgaris* was constantly present, sometimes in sufficient numbers to be deemed the dominant form; *Centropyxis aculeata* appeared once only but in large numbers; the others were less numerous but almost always to be found.

In these filters the phenomenon of sporadic dominance on the part of any one species was far less marked. Certain species, such as *Aeolosoma* sp., *Macrobiotus* sp. and *Cænomorpha medusula*, were found in fair numbers and on only one occasion in each case, but there was not the violence associated with such changes in the first filters. Ecologically, the second filters were typical of the beta division of the mesosaprobic zone.

Brent River.

The river (a local euphemism for "brook") was sampled above and below the points at which the two effluents discharged into it. The change in biological population was relatively slight, but sufficiently marked to be noticeable even without quantitative measurements. Above the effluents, unicellar and filamentous algæ of many kinds were very plentiful, especially the crescentic form *Closterium* sp. *Stentor cæruleus* was also very common, and *Stentor polymorphus* less so. *Arcella vulgaris* and *Aspidisca costata* among the protozoa and the rotifer *Philodina* sp. may also be included among the principal organisms.

Below the effluents the most noticeable point was the great reduction in the numbers of chlorophyllaceous forms, and the absence of the two stentors. *Aspidisca* was still common, and was joined by vorticellids, *Lionotus* sp. and *Colpidium* sp. It may be assumed that "Brent B" was largely responsible for this change, though "Brent A" would be a contributory cause at the time of the sloughing of the film from the second filters, since there is no final humus tank on this side.

EAST HAM.

The general remarks about physico-chemical processes made above in relation to Brent apply equally to East Ham. The accompanying table (II) will show that the pH.-values and oxygen values are of an order comparable with those of Brent. Certain features here, however, are worthy of note. The bio-aeration tank ("Plant A") effectively oxidises a raw sewage with a 10-minute oxygen demand of over 30 mgm. per litre to an effluent with a demand of about 5 mgm. Owing to the use of efficient Dortmund tanks this effluent is remarkably clear and free from suspended solids. But then the less efficient process of chemical precipitation, and filtration through stones by means of fixed nozzles,

(" Plant B ") which discharges its effluent together with that of " Plant A " into a common humus tank, has the effect of raising the oxygen demand again to about 8 mgm. per litre. It will be noticed that the sample from the chemical precipitation tank already shows a considerable reduction in oxygen demand ; the tank had been standing for

East Ham, 27/11/28.

Location.	pH.	Oxygen.	Notes.
Influent, Aeration Tank ...	*	32·0	Bio-Aeration Process.
Effluent, Aeration Tank ...	7·55	5·8	
Effluent, Dortmund Tank...	7·75	5·0	
Chemical Precipitation Tank	*	20·0	Precipitation and Filtration Process.
Final Effluent	7·5	8·0	Common to Both Processes.

* Owing to extreme opacity no determinations were made.

Table II.—Physico-chemical data of East Ham Sewages. For explanation see text.

some time, and much of the suspended material had evidently settled out. Another noticeable point is the significant pH.-difference between the aeration tank effluent and the Dortmund tank effluent. These liquors are essentially the same except that in the latter case the solids have settled out ; it seems probable that some of the particles must have carried down positive electric charges with them.

The fauna of the bio-aeration process is mostly contained in the flocs of activated sludge which are carried through the aeration tank, settle in the Dortmund tanks and are pumped back to the aeration tank again. It was found to be curiously limited as regards numbers of species. There were hosts of minute flagellates and ciliates, and bacteria of the vibrion, coccus and streptococcus forms, on all occasions. Marked changes in dominance were also apparent. On one occasion *Opercularia berberina* was the dominant form ; on another it was *Aspidisca costata*. A quantitative estimate of part of a floc showed that this rhizopod was present at the rate of 12,500 per cubic centimetre. Among other

less numerously represented genera were: *Vorticella* (several spp.), *Chilodon*, *Cyclidium*, *Chænia*, *Vahlkampfia*, *Chlamydomonas*, *Notosolenus*, *Anthophysa* and *Paramœcium*. On one occasion a single nematode was found (*Diplogaster* sp.) and on another a single *Cyclops*. In the supernatant liquid of the Dortmund tank ("Plant A") and of the chemical precipitation tank ("Plant B") very few organisms were to be found; in both cases they would be carried to the bottom with the settling sludge.

COMPARISON OF BRENT AND EAST HAM.

The accompanying figure (2) shows the oxygen absorption curves for fairly typical final effluents from the three plants, "Brent A," "Brent B" and East Ham ("A" and "B" combined). As regards efficiency they would seem to stand in the order stated. Certainly the "Brent A" effluent is superior to that of "Brent B," and the indications are that the "East Ham A" effluent alone is also purer than "Brent B." But there is a difficulty in comparing the sewage liquors from the two localities, since they necessarily differ in constitution. For instance, the East Ham raw sewage is not only stronger but also far more opaque than the influent to the filters at Brent. Moreover, the oxygen absorption curves for samples from the two locations reveal a marked difference in type. This is illustrated in figure 3 which shows typical curves for the final effluent of "Brent B" and for the effluent of "East Ham A" alone, respectively, these two liquors being of a comparable order. The East Ham curve is decidedly steeper than the Brent one for the first six minutes of absorption, after which it is flatter. Any single pair of oxygen values selected, up to an absorption time of ten minutes, would convey the impression that the Brent effluent was the purer one, whereas a consideration of the curves in their entirety leads to the reverse conclusion. It appears that the Brent effluents are slower in taking up oxygen than those of "East Ham A." This point indicates that, short of the lengthy determination of total oxygen demand, isolated titration values should be received with caution; it also shows that they may be less informative, though more accurate, than curves obtained by the methods here described.

In so far as any biological comparison of the two plants is justifiable, the more limited fauna of "East Ham A" would seem to indicate a higher degree of saprobic conditions. But, while at Brent any given

colony of organisms in the filters is continuously immersed in a medium of practically constant oxygen demand, a like colony in a floc of activated sludge at East Ham is repeatedly carried round the tank from regions of very high, to those of very low, oxygen demand. Under such diverse conditions comparison is of little use.

FIG. 2.

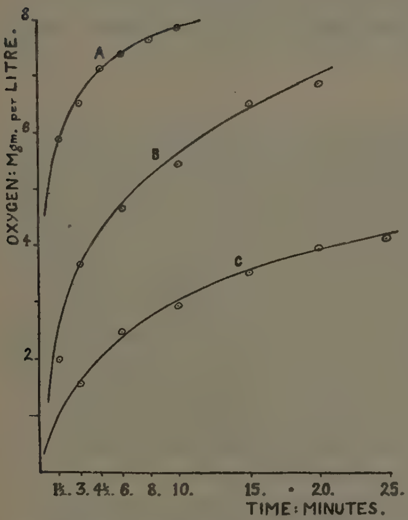


FIG. 3.

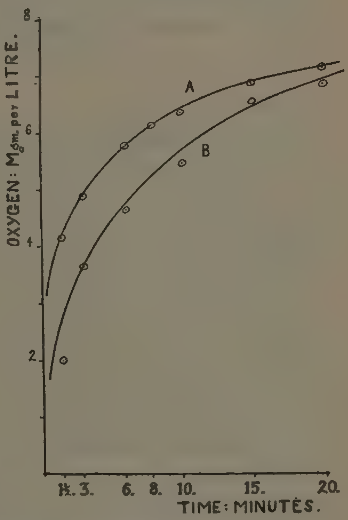


Fig. 2.—Curves showing the Oxygen Absorption of the Final Effluents from :—A, East Ham ; B, " Brent B " ; and C, " Brent A ".

Fig. 3.—Curves showing the Oxygen Absorption of two comparable samples from :—A, " East Ham A " ; and B, " Brent B ", respectively.

Considering together all the units sampled from all plants, the writer has analyzed the data to show the distribution of each key-organism throughout the various units. Thus a category which might be termed the " ubiquity " or " dispersal " of the different species is obtained.

The analysis shows *Vorticella* sp. to be the most ubiquitous, occurring as it does in 11 units. Next come *Aspidisca costata* and *Opercularia berberina* with 7 units each. For the rest, there are several in each category from 5 to 1 units. The most ubiquitous of the metazoa was the rotifer *Philodina* sp.

Miscellaneous Notes.

Under this heading are collected notes on certain points which, while strictly extraneous to the main thesis, have yet an indirect bearing upon it.

THE FLORA OF SEWAGE.

Bacteria of various types have, of course, constantly been found in the sewage samples, but no attempt at diagnosis or estimation has been made by the writer. Various workers have published data on this point, however. Bell (1927), working on the film around the stones of sewage filters, found the principal bacteria to be *Bacillus subtilis* and *B. coli communis*. In a paper on " Anaerobes in Sewage," Greer (1926) states that the most common are *Clostridium welchii* and *C. sporogenes*, while less common are *C. tetani* and *Vibrion septique*. With special reference to activated sludge, Ruchhoft & Watkins (1928) find that most of the filamentous bacteria in the flocs of sludge belong to the genus *Sphærotilus*. *Beggiatoa* is reported by numerous observers.

As regards the fungi, Bell (1927) working on Barnsley sewage found that the two principal forms were the mould *Oidium lactis*, and the pink wild yeast *Torula rosea* ; the moulds *Mucor mucedo* and *Penicillium* sp. were also found. At New Jersey (1923) species of *Oidium*, *Penicillium*, *Pythium* and *Dictyuchus* have been recorded.

From the last mentioned source is a record of the algæ *Stigeochonium* and *Oscillatoria*, which are said to have been common on the surface of the filters. The workers at New Jersey have an experimental filter so arranged that samples can be taken throughout its depth. They were thus able to record that the algæ are found only on the surface, where the fungi are relatively rare. These appear below the surface but in the upper part of the filter, while the filamentous bacteria occur throughout.

ACHORUTES VIATICUS.

The inoculation of filters with the eggs of the springtail *Achorutes viaticus*, in order to prevent or reduce ponding, is recommended by many authors (*e.g.*, Bell, 1922, and Mitchell, 1928). In a later paper Bell (1927) shows that the insect actively feeds on the fungi present. The filamentous bacteria, fungi and algæ (noted in the previous section) form a more or less dense network throughout the filter, and if this becomes too dense aeration and the free passage of sewage are retarded. Apparently the springtails on the surface and their larvæ in the sub-surface reduce this danger by feeding on at least the fungi. *Achorutes* has been found at Brent, but not in large numbers.

NEMATODES IN SEWAGE.

Reference has already been made (page 141) to the influence of protozoa in controlling the increase of bacteria in sewage by devouring them in large numbers. Purdy (1926) records results of experiments with *Paramœcium* which show the same thing; bacteria alone increased enormously; with added protozoa they were greatly reduced; protozoa free from bacteria ceased to live. It is highly probable that the nematodes play a similar rôle. Thus Zimmermann (1921) has proved that the vinegar eelworm will not live in sterile vinegar, even killed cultures of bacteria being insufficient to sustain it. Certain of the free living nematodes, however, are definitely predaceous, pursuing other nematodes, killing them, and extracting their juices by means of a powerful sucking apparatus. The nematode *Diplogaster striatus*, which occurred at Brent, is an example. The writer has found this worm attacking the rotifer *Philodina* by means of its buccal armature and later has seen the fluid contents of the rotifer rapidly passing down the œsophagus of the worm. It is impossible to classify such predatory forms as either harmful or beneficial. The bacterivorous forms are essential, but they must not increase unduly. A Rothamsted report (1925-26) records the failure of a sewage plant; yeast, introduced into the sewage from a brewery, stimulated the protozoa which then "Reduced the bacterial population so much that they could not adequately purify the sewage." In this case the predatory nematodes would have been distinctly beneficial. Efficiency requires a nice balance between bacteria and bacterivorous forms, and predatory species constitute a definite factor in that balance.

The nematodes display a constant and rapid snake-like movement which has a considerable physical influence on the zooglœal film of the filters. Being considerably smaller than the commoner oligochætes they do not appear to loosen the film sufficiently to cause it to slough off. Their action in this respect seems rather beneficial than otherwise, since they tend to prevent too close a matting together of the minute filamentous organisms that bind the film together. This induces more efficient aeration and facilitates that intimate contact of the film with the percolating sewage which is the essence of the filtration process.

References.

Bell, H. D., 1922.—"The Maintenance of Clean Filtering Medium in Sewage Filters." *Trop. Dis. Bull.*, Suppl. 1, p. 29. (W.L. 21797.)

— 1927.—"Research on the Composition of the Grey Slimy Growth on the Surface of Sewage Filters." *Surveyor Lond.*, lxx, 561-565. (W.L. 20782.)

Buswell, A. M., 1928.—"The Chemistry of Water and Sewage Treatment." 1-362. (New York.)

Clark, W. M., 1925.—"The Determination of Hydrogen Ions." 1-480, 2nd Edit. (Baltimore.)

Cohn, M. M., 1925.—"Lime Hypochlorite lowers Psychoda Flies at Sewage Works." *Eng. News Rec.*, xciv, 684-685. (W.L. 7842.)

ENSLOW, L. H., 1927.—" Recent Developments in Sewage Chlorination," from " Fly Nuisance and Filter Pooling." (Texas.)

FAIR, G. M., & CARLSON, C. L., 1927.—" Hydrogen Ion Control of Sludge Digestion." *Eng. News Rec.*, XCIX, 881-883.

GREER, F. E., 1926.—" Anaerobes in Sewage." *Amer. J. Publ. Hlth.*, XVI, 500-501. (W.L. 617.)

HEUKELEKIAN, H., 1927.—" The Fate of B. coli and B. aerogenes in Sewage Purification." *J. Bact.*, XIV, 55-67. (W.L. 11056.)

KERSHAW, G. B., 1925.—" Sewage Purification and Disposal." 2nd Edit., xii + 364. (Cambridge.)

KOLKWITZ, R., & MARSSON, M., 1909.—" Œkologie der tierischen Saprobien." *Int. Rev. Hydrobiol.*, II, 126-152. (W.L. 10162.)

MARTIN, A. J., 1927.—" The Activated Sludge Process." xiv + 415. (London.)

MITCHELL, W. A., 1928.—" Glossop—Sewerage and Sewage Disposal." *Proc. Instn. Munic. Co. Eng. Lond.*, LV, 266. (W.L. 16771.)

NEW JERSEY, 1923.—" Studies on the Biology of Sewage Disposal." *Bull. New Jers. Agric. Expt. Stas.*, No. 390, 1-78. (W.L. 4634.)

— 1924.—" A Bacteriological Study of a Sewage Disposal Plant." *Ibid.*, No. 394, 1-24.

— 1925.—" Studies on the Biology of Sewage Disposal." *Ibid.*, No. 417, 1-39.

— 1926.—" Studies on the Biology of Sewage Disposal." *Ibid.*, No. 427, 1-103.

PETERS, B. G., 1928.—" Some Nematodes of a Sewage-disposal Plant," an otherwise unpublished section of a Thesis, " *Saprophytic Eelworms*," submitted to the University of London, 20 pp.

— 1929.—" A New Wedge-colorimeter used in a Biological Investigation of Sewage." *J. Helm.*, VII, 202-214.

PURDY, W. C., 1926.—" The Biology of Polluted Water." *J. Amer. Wat. Wks. Ass.*, XVI, 45-54. (W.L. 11023.)

PURVIS, J. E., 1926.—" The Influence of Hard and of Soft Waters on the Decomposition of Sewage." *Surveyor Lond.*, LXIX, 277-278.

ROTHAMSTED EXPERIMENTAL STATION, 1927.—" Abridged Report for 1925 and 1926." pp. 39 and 40.

RUCHHOFT, C. C. and WATKINS, J. H., 1928.—" Bacteriological Isolation and Study of the Filamentous Organisms in the Activated Sludge of the Des Plaines River Sewage Treatment Works." *Sewage Wks. J.*, I, 52.

RUDOLFS, W., 1927.—" Sewage Investigation at the New Jersey Agricultural Experiment Station." *Proc. 9th Texas Wat. Wks. Short School*, 352-355.

SAUNDERS, J. T., 1924.—" The Effect of the Hydrogen Ion Concentration on the Behaviour, Growth and Occurrence of *Spirostomum*." *Proc. Cambridge Phil. Soc.* (*Biol. Sci.*), I, 189-203.

WHIPPLE, G. C., 1927.—" The Microscopy of Drinking Water." 4th Edition Revised, xix + 586, 19 plates. (New York.)

WIGLEY, C. G., 1926.—" Progress Report of the Special Committee of the Sanitary Engineering Division on Co-operation with the New Jersey Sewage Experiment Station." *Proc. Amer. Soc. Civ. Eng.*, LII, 306-310. (W.L. 16634.)

Wilson, J. A., 1924.—" Colloid Chemistry as Applied to Activated Sludge." *Intern. Conf. Sanit. Eng., London* : quoted in Johnston, 1924.—" Reports of the Progress of Applied Chemistry." *Soc. Chem. Ind.*, ix, 1-20 (W.L. 1465.)

Zimmermann, A., 1921.—" Recherches expérimentales sur l'Elevage aseptique de l'Anguillule du Vinaigre." *Rev. suisse Zool.*, xxviii, 357-380. (W.L. 19288.)

Journal of Helminthology, Vol. VIII, No. 3, September 1930, pp. 165-184.

Some Nematodes met with in a Biological Investigation of Sewage.

By B. G. Peters, M.Sc., Ph.D.

Research Scholar in Sanitary Science of the Grocers' Company, 1927-1929.
(From the Department of Helminthology, London School of Hygiene and Tropical Medicine.)

Introduction.

This is the third and final paper of a series which constitutes a report on a biological investigation of sewage, undertaken by the writer as a Research Scholar of the Grocers' Company. Of the previous two papers, the first (1929) describes a colorimeter and the second (1930) is a general report on the work as a whole. Since the nematodes form a neglected and not inconsiderable section of the fauna of most sewage plants, special attention was accorded them, and an account of them is given separately here instead of rendering the general report unbalanced and unwieldy by being included there.

The various species described were found living in the zoogloeal film which surrounds the stones of the sewage percolators at Brent. Notes on their distribution and biology appear in the general report (1930). The account of *Diploscapter coronata* is quoted from a paper (1928) submitted to the University of London as part of a thesis for the Degree of Ph.D., but hitherto unpublished in any journal; the remainder of the material appears here for the first time.

With regard to this work on the nematodes, the writer would like to express his thanks to Professor R. T. Leiper, F.R.S., under whose supervision the Scholarship was held, for suggesting this line of investigation and for his interest and assistance—particularly in making accessible his collection of helminthological literature.

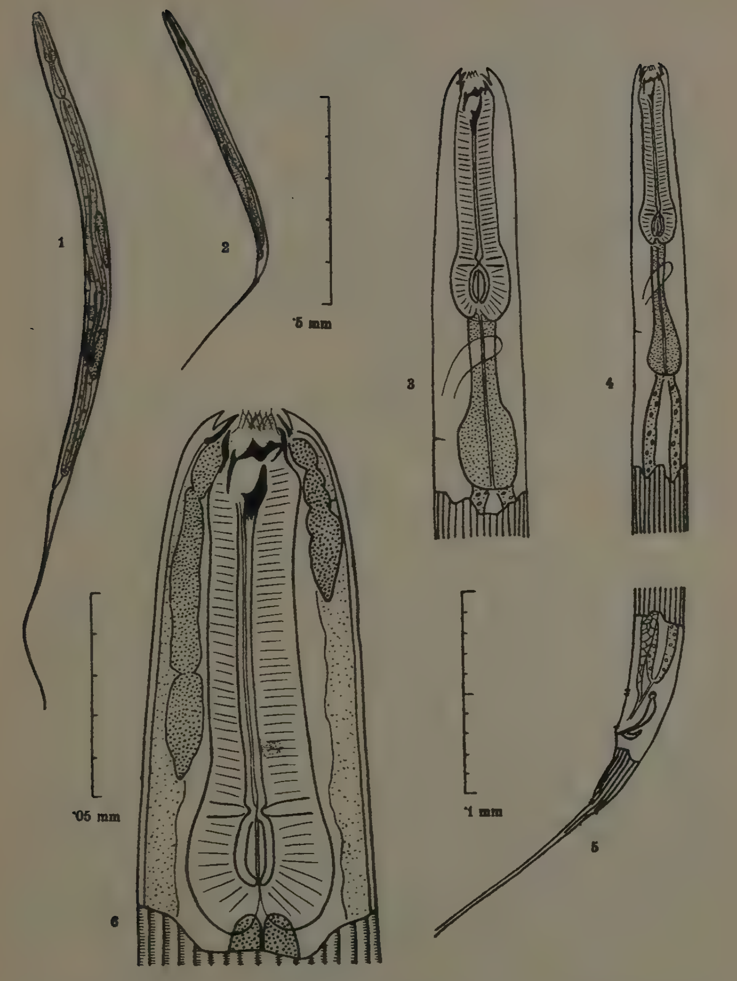
Diplogaster striatus Bütschli, 1876.

On the whole, the diplogasters were the commonest nematodes met with in the sewage samples. In the course of examinations many drawings

were made and put aside for subsequent consideration, as a result of which it was found that many species were present. Of these, the most interesting was a predaceous species which was found actively attacking other nematodes and rotifers, penetrating their integuments with its large dorsal tooth and then sucking in the fluid contents of their bodies. This species is figured opposite. Next to the formidable mouth armature, the most striking features of this worm are the deep longitudinal ridges with which the entire body is furrowed, and the long filamentous tail. The most common species with these longitudinal furrows are *D. liratus*, *D. nudicapitatus* and *D. striatus*. Of these, *D. liratus* (Schneider, 1866) has three cuticular thickenings in the mouth instead of a single large tooth, it is less than one-third the length of the present species, it is relatively much stouter, its œsophagus is longer and tail much shorter. While *D. nudicapitatus* Steiner, 1914 apparently has a large tooth, it is lacking the forwardly directed processes to be found in the present species (fig. 6), its spicules are more slender than the accessory piece, it is less than half the length of these sewage worms and its œsophagus is relatively longer and tail shorter. Of the three, *D. striatus* Bütschli, 1876 is most like the present species, which I have assumed it to be.

A formula descriptive of the proportions of a given worm has been worked out by de Man. Once the significance of his ratios has been grasped, they form a useful basis for the comparison of one worm with another. He gives first the total length, and the the ratio of the total length to the width at the middle of the body (represented by the letter alpha), then the ratio of the total length to the length of the œsophagus, including the mouth (represented by beta), and finally the ratio of the total length to the length of the tail (represented by gamma). Micoletzky usually adds a value denoting the distance of the vulva from the anterior end expressed as a percentage of the total length (represented by V), this notation conveying at a glance whether the vulva is in the posterior or anterior part of the body.

In giving the principal dimensions I have added such values as are recorded for *D. striatus* by Bütschli himself (1876) and by Micoletzky (1921). The average length of the female is 1·74 mm. (Btsch, 1·5 mm. ; Micol. 0·82 mm.) and of the male 1·0 mm. (Btsch, 1·0 mm., Micol,



Diplogaster striatus. Fig. 1, female. Fig. 2, male. Fig. 3, anterior end of female. Fig. 4, anterior end of male. Fig. 5, posterior end of male. Fig. 6, head of female. (All lateral view.)

0·66 mm.). The ratio a is for the female 28 and for the male 25 (Micol, common value : 26). The ratio β is for the female 8·1 and for the male 6·4 (Btsch, common value : 7 to 8 ; Micol : 7·5 and 6·3 respectively). The ratio γ is for the female 3·1 and for the male 3·0 (Btsch., common value : 3 to 4 ; Micol. : 3·2 and 3·5 respectively). The percentage V is 40 (Btsch. : " in der mitte " ; Micol. : 41). It will be noted that Bütschli's specimens are very much longer than those of Micoletzky, and that my specimens are longer still ; it seems obvious that considerable variations in size are possible. Apart from this, the various ratios are well in agreement. Nevertheless, there is a real danger of these ratios being misleading in the case of any species having a long filiform tail, for the tail is apt to be broken ; this danger would be removed if the ratios could be calculated on the length from anterior end to anus instead of on the total body-length.

The buccal armature (fig. 6) is very complicated and difficult to interpret. The entire cavity is lined with cuticularized plates and is about 0·015 mm. deep. The mouth is surrounded by a number of conical papillæ (apparently 6) each of which is surmounted by a small bristle. The dorsal tooth extends across to the ventral side, is irregular in outline and appears to rest on (rather than to be fused with) a stout peduncle which springs from the dorsal wall lining the lumen of the œsophagus. The base of the peduncle has a ventral spur. Bütschli figures a tooth that resembles an equilateral triangle in shape and appears to be fused with a quite slender peduncle. Moreover, in his figure, the tooth does not extend half way across the buccal cavity. He figures no crown of papillæ, but about 10 ribs in their place. Between the œsophagus and the body wall anteriorly there are a small dorsal body and a longer ventral body which appear to be glandular in constitution (fig. 6.). The muscular portion of the œsophagus is stout and terminates in a slight bulb containing a valvular apparatus of the typical diplogastroid type, the lumen being occluded by the pressure of the walls. In both sexes this muscular section extends for about 60 per cent. of the total length of the œsophagus. The posterior section is non-muscular and expands into a bulb before opening into the intestine.

The female gonads are paired, opposed, reflexed and symmetrical, extending about 0·2 mm. on either side of the vulva. There is only

one egg in each uterus at a time, the worm being oviporous. The male gonad is single and outstretched, and extends about 0·3 mm. from the ano-genital pore. The spicules are arcuate and bear knobs distally; they are about 0·032 mm. long. There is a well-marked accessory piece. Of the male papillæ two are pre-anal and ventral and one ad-anal. There is a group of three small papillæ ventrally and one dorsally at the point where the body narrows suddenly to form the tail. The latter is always flexed anteriorly.

About 15 longitudinal ridges are visible at a time in the female and 12 or 13 in the male. I have shown these in the terminal portions of figures 3 to 6. Under high magnifications (fig. 6) transverse striations are visible on the crests of the ridges; these are rather less than one micron apart. Excretory pore and nerve ring are normal in position.

Other diplogasters have been found, including another form with marked longitudinal striations but smaller and with a far simpler mouth armature, quite probably *D. liratus*, and other species lacking longitudinal striations. But the material of any single species is insufficiently complete to afford grounds for discussion.

Diploscapter coronata (Cobb, 1893) Cobb, 1913.

This nematode appeared quite suddenly in the percolators in January, 1928, and disappeared again with equal abruptness, a phenomenon not unusual in a medium of a highly saprobic character. Though actively sought after, no males have been found at any time.

On superficial examination the worm is seen to be small (less than half a millimetre in length) and relatively stout, and to display the writhing in a horizontal plane so characteristic of free-living nematodes; the movements, however, are noticeably sluggish. The œsophagus has a well-marked posterior bulb containing a valvular apparatus, and is connected by a narrow neck to a slightly distended anterior portion which, in many specimens, scarcely deserves the name of anterior bulb. Anteriorly there is a cylindrical or prismatic pharynx. Thus the worm bears a close resemblance to the species of *Rhabditis*, to which genus I first thought it to belong. However, there are one well-marked feature and one less obvious, in the anterior region, which are not typical of that genus: the dorsal and ventral lips are strongly cuticularized

in a peculiar way, and the posterior end of the pharynx is abrupt and clean. In *Rhabditis* the pharynx appears as a pair of rods, and immediately behind each of these is the appearance of a small nodule of similarly cuticularized material. Micoletzky (1921) has compared this appearance, when the worm is viewed with the head away from the observer, with a mark of exclamation (!) ; I believe these nodules to be two optical projections of a single ring, since the same appearance obtains in a ventral view of a worm as in the usual lateral view. In any case, this ring or group of nodules has not been found in the worms in question ; and its absence, together with the curious lips, suggested that the worms could not be placed in *Rhabditis*.

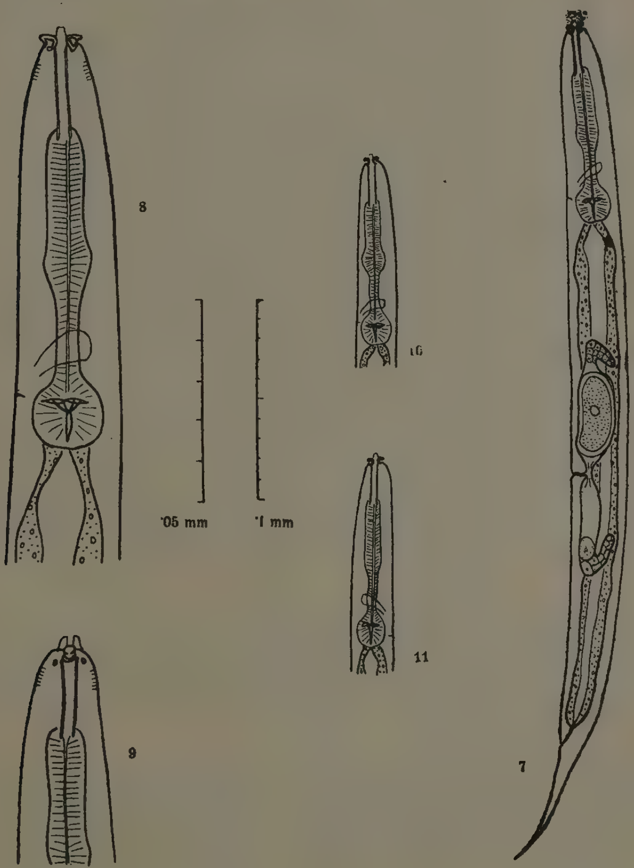
A search through Micoletzky's paper and through Baylis and Daubney's " Synopsis " revealed the unispecific genus *Diploscapter* as the probable designation of the worms, and subsequent measurements have confirmed this.

Historical.

In 1893 Cobb described a new species of nematode, *coronata*, which he placed in the genus *Rhabditis*. He gave his usual " formula " for the worm together with a short description and some small and rather vague drawings, based on a single specimen, a female. " The head," he said, " was surmounted by six (?) conical lips each turned outwards." I have been able to distinguish only four lips, from lateral and ventral views, in the worms from the sewage filters, and my worms had an anterior œsophageal bulb far less marked than that in Cobb's drawing ; but his formula fitted very closely, he was himself doubtful about the six lips, and the other features of his worm and mine were very similar. His single specimen was found " In humus about the roots of banana plants, Fiji."

In 1913 Cobb published a short note on a new genus, *Diploscapter*, " Established to receive the writer's *Rhabditis coronata*." The note gave a wider distribution to the worm, stated " Possibly more than one species may be represented in the descriptions of the various authors," and contained a revised formula for the female together with one for the male. No mention was made of the lips, and it is not clear whether the formulæ were based on new material which had come into his hands or on the measurements of other authors.

Meanwhile three other papers had appeared on this worm: de Man, 1896, Zimmermann, 1898, and Maupas, 1900, the first and third of which I have consulted. De Man found the worm, together with a new species



Diploscapter coronata, female. Fig. 7, entire worm (lateral view). Fig. 8, anterior end (lateral view). Fig. 9, anterior end (ventral view). Figs. 10 and 11, illustrating variations in anterior œsophageal bulb (lateral view).

of *Aphelenchus* and a new species of *Rhabiditis*, in diseased pseudobulbs of a tropical orchid (*Calanthe* sp.). " Though very many female individuals were found in the humus-like substance of the diseased *Calanthe*," he says, " only one single male specimen was observed by me." He describes four lips, the dorsal and ventral of which are heavily cuticularized and reflexed outwards. His measurements will be considered later together with those of Cobb, Maupas and Micoletzky.

Zimmermann apparently described a new species, *Rhabditis bicornis*, which Micoletzky regards as identical with *Diploscapter coronata*.

Maupas' paper on types of reproduction in nematodes contains a good description of the worm, with figures. He finds no trace of the striations noted by Cobb and figured by de Man in the anterior region of the worm, and states that a median œsophageal bulb cannot plainly be distinguished—which statement does not agree with Cobb's original figure. The paired uteri never contain more than one egg between them, the eggs being laid before the first cleavage occurs.

Maupas isolated 150 immature larvæ all of which became females laying fertile eggs which developed normally; the uteri of the worms contained spermatozoa identical with those of a male. " There can be no doubt that the species is an autogamous protandrous hermaphrodite." The ratio of males to females in Maupas' cultures was 5 or 6 per 1,000. He considered the males to be non-functioning.

Morphology.

A brief description will now be given, with figures, of the specimens obtained from the Brent trickling filters, after which the data of other authors will be compared with a view to considering the significance of the variations observed.

The length of mature females varies only slightly around 0·4 mm., and the breadth around 0·024 mm., the worm figured (fig. 7) being a relatively large one. The head is rather abruptly rounded while the tail tapers gradually to end in a fine point. Over the greater part of the body striations can not be seen, but in the region of the pharynx transverse striations (about 1 micron apart) are visible with the highest powers at the margins of the body. Thus it can be understood why Cobb describes the worm as striated, while Maupas denies the presence

of striations. The lateral fields are plainly visible and unusually wide, the width being 0·005 mm., or about 1/5th of the body diameter.

The mouth is guarded by four lips which constitute the chief diagnostic character of the species. At low magnifications and from a lateral aspect the dorsal and ventral lips appear as a pair of outwardly turned crescents (fig. 7), composed of highly cuticularized material; at higher magnifications (fig. 8) the ends of each crescent are seen to be connected by a bar of less dense material, the lip now resembling a distorted triangle. These lips are about 4·5 microns in length. The pair of lateral lips is less easy to see, partly because they are composed of very transparent material and partly because every specimen examined carried a plug of adventitious matter in its mouth (fig. 7). A ventral view, however, clearly displays the lateral lips as a pair of forwardly directed processes about 4 microns in length (fig. 9). Although I have observed these worms closely, I have never seen the dorsal and ventral crescentic processes moving in any way. It has been suggested that they are used as a sort of digging instrument by being forced outwards; this would be difficult in view of the fact that the lateral processes (for which no movement is claimed) project some distance in front of the others.

The mouth leads directly into a pharynx which appears to be cylindrical rather than prismatic, and which extends about 0·02 mm. from the mouth to end abruptly in the substance of the œsophagus. It is about 0·003 mm. in diameter.

The œsophagus presents three well-marked portions: an anterior cylindrical portion about 0·035 mm. long and 0·009 mm. wide, a narrower neck about 0·025 mm. long and 0·005 mm. wide, and a roughly spherical bulb about 0·017 mm. in diameter. The bulb contains a well developed valvular apparatus of the rhabditid type, and the whole œsophagus is muscularized.

The posterior end of the first portion of the œsophagus shows, in most specimens, a dilatation which has been referred to as the anterior bulb by previous authors. Cobb (1893) figures a very marked spherical bulb in this position; Maupas states that a median bulb cannot clearly be distinguished. I have found great variations in the size of this dilatation, some specimens scarcely showing it at all, as will be seen from the figures (figs, 7, 10, 11), and I conclude that it is not a constant feature.

The nerve ring crosses the neck of the œsophagus immediately in front of the posterior bulb. The ventral excretory pore is visible at the level of the anterior end of the valvular apparatus. The intestine calls for no special note.

The vulva occurs just behind the middle of the body in the ventral line, its position being very constant at a point about 54 per cent. of the body-length as measured from the head. There are paired opposed short uteri, succeeded by very short reflexed ovarian tubules, the entire reproductive apparatus giving the impression of being immature. Usually only one unsegmented ovum is present at one time, sometimes in the posterior sometimes in the anterior uterus ; Maupas and Micoletzky both state that the uteri never contain more than one ovum at a time, but this is not a diagnostic feature since one of my specimens revealed two full-sized ova, one in each uterus. The reproductive apparatus extends only about 0·048 mm. either side of the vulva (de Man gives the length as two-thirds of the distance between the vulva and the posterior end of the œsophagus, which would be about 0·08 mm. from the vulva), and the ova measure 0·044 × 0·016 mm., so there is obviously not room for more than one ovum in each uterus. De Man gives 0·043 to 0·051 mm. as the length of the ova, and the breadth as half as much ; Maupas gives the dimensions as 0·052 × 0·02 mm. ; it is clear, from de Man's values, that considerable variations are possible, and it is natural that the unusually short uterus of my specimens should contain unusually small eggs.

Variations.

Variations in the measurements of the different authors can now be considered. For this purpose the Cobb formula has been worked out for my specimens, as well as the de Man formula, and Cobb's and Maupas' data have been translated into the de Man formula for general comparison.

Cobb's first paper (1893) contains the following formula :—

$$\frac{6{\cdot}0 \quad 17{\cdot}0 \quad 26{\cdot}0 \quad 55{\cdot}0 \quad 81{\cdot}0}{4{\cdot}0 \quad 4{\cdot}8 \quad 5{\cdot}2 \quad 5{\cdot}8 \quad 2{\cdot}8} \quad 0{\cdot}36 \text{ mm.}$$

The figures in the upper row are the distances from the anterior end, of the base of the pharynx, the nerve ring, the posterior end of the posterior œsophageal bulb, the vulva, and of the anus respectively ; the

corresponding values below are the body-diameters at those points: all measurements are in percentages of the total body-length, which terminates the formula.

Cobb's second paper (1913) contains a revised formula for the female, together with one for the male which will not be considered here. The revised formula, shorn of certain additional symbols, is as follows :—

$$\frac{4{\cdot}5 \quad 16{\cdot}5 \quad 24{\cdot}2 \quad 55{\cdot}0 \quad 89{\cdot}5}{4{\cdot}0 \quad 5{\cdot}4 \quad 6{\cdot}1 \quad 6{\cdot}3 \quad 2{\cdot}8} \; 0{\cdot}4 \text{ mm.}$$

The corresponding Cobb formula for my specimens, giving average values from seven selected adult females, is :—

$$\frac{6{\cdot}03 \quad 18{\cdot}34 \quad 24{\cdot}04 \quad 54{\cdot}0 \quad 86{\cdot}5}{4{\cdot}11 \quad 5{\cdot}22 \quad 5{\cdot}56 \quad 5{\cdot}97 \quad 2{\cdot}30} \, 0{\cdot}41 \text{ mm.}$$

Maximum and minimum values have also been worked out for my specimens, as follows :—

$$\frac{(5{\cdot}4\text{–}6{\cdot}4)(16{\cdot}8\text{–}19{\cdot}7)(22{\cdot}5\text{–}25{\cdot}5)(52{\cdot}0\text{–}56{\cdot}0)(84{\cdot}0\text{–}90{\cdot}0)}{(2{\cdot}8\text{–}4{\cdot}8)(\ 5{\cdot}0\text{–}\ 5{\cdot}6)(\ 5{\cdot}3\text{–}\ 6{\cdot}0)(\ 5{\cdot}1\text{–}\ 6{\cdot}3)(\ 1{\cdot}7\text{–}\ 2{\cdot}8)} \, 0{\cdot}38\text{–}0{\cdot}46 \text{ mm.}$$

An examination of these values will show that there are no variations of significance as between Cobb's specimens and mine.

De Man's formula is widely used, and is less involved than that of Cobb. The symbols have already been explained on page 166. As calculated from the dimensions given by each of the various authors the de Man formulæ are as follows :—

Author.	*Length.*	α.	β.	γ.	*V.*
Cobb (1893) ...	0·36	17·2	3·9	5·3	55%
Cobb (1913) ...	0·4	15·9	4·1	9·5	55%
De Man ...	0·6	16–17	4·86	7·2–8	50%+
Maupas ...	0·5	15	5	7	53%
Micoletzky ...	0·35–0·6	14–17	3·7–5	7–9·5	50%+
Peters—					
Average ...	0·41	16·9	4·1	7·6	54%
Maximum ...	0·46	19·6	4·4	9·0	56%
Minimum ...	0·38	15·9	3·9	6·3	52%

My own values give the average for seven specimens, the maximum and the minimum. Considering these together with those of the previous authors, it will again be seen that there are no variations of sufficient

significance to warrant the creation of new species. I conclude that all these forms are correctly to be designated *Diploscapter coronata* (Cobb, 1893) Cobb, 1913.

Dorylaimus saprophilus spec. nov.

While *Diploscapter coronata* was the smallest nematode met with in the sewage samples, the present worm was by far the largest, being over ten times the length of *D. coronata*. In general appearance (fig. 12) it is seen to be a worm over 4 mm. in length; as compared with this length the body is fairly slender, the œsophagus rather long, and the tail very short with its extremity slightly flexed ventrally. The anterior end tapers gradually ending in a rather blunt head, the buccal spear being plainly visible. The intestine is brown in colour and stands in marked contrast to the pale yellowish prerectum posteriorly. Oblique anal muscles are very obvious. The vulva shows distinctly, but the paired ovarian tubes are clearly visible only at their extremities where they double back on themselves and displace the intestine somewhat. No males have been discovered, and if they had been present they could not easily have been overlooked in view of the large size of the species. However, in many existing species of *Dorylaimus* the male is unknown. Moreover, I was unable to find immature forms in the samples collected.

Morphology.

The cuticle has a considerable thickness, varying from 0·005 mm. at the extreme anterior end to 0·015 mm. towards the posterior end, and constitutes entirely the posterior 0·04 mm. of the tail. It is crossed irregularly by minute canals, particularly in the posterior region, which pass from the body cavity in a slightly oblique direction posteriorly from within outwards. No transverse or longitudinal striations are visible. Surrounding the small mouth are six large, rounded, protuberant lips, each bearing a distinct labial papilla. Viewed from the ventral aspect (fig. 16) the lips appear to be evenly continuous with the body wall behind. But from the lateral aspect (fig. 17) they are seen to be separated from it by a constriction. Most of the drawings were made from specimens killed by heat, but part of the material was killed with Ditlevsen's fixative and left in weak glycerine alcohol for about 50 days, after which they were stored in pure glycerine. As a result of this

treatment it was found that the lips protruded even more so (fig. 18), appearing quite distinct from the body behind. Moreover, in one of the treated specimens there was, dorsally and ventrally, a small bristle-like papilla extending from the cleft between the head and the body. The inner surfaces of the lips pass backwards for a distance of about 0·03 mm. to form the narrow buccal cavity. From the base of this cavity there springs forward the small basket-like device (figs. 16, 17, 18) which is said to be a guide for the spear. The basket is about 0·005 mm. long and about 0·01 mm. in greatest width, and consists principally of two rings.

When the spear is retracted it extends back about 0·1 mm. from the anterior extremity, and rests in the basket with the point lying among the lips. On a ventral view the hollow spear is seen to be cut obliquely at the anterior end (fig. 16) like the end of a goose-quill. For the greater part of its length it is about 0·006 mm. in diameter, and has stout, heavily cuticularized walls; but at about half its length the diameter and the thickness of the walls increase irregularly, and in this region there seems less cuticularization. Posteriorly the lumen of the spear is continuous with that of the œsophagus.

At a point near the thickening of the retracted spear the œsophagus arises (fig. 13), and passes back to a distance of about 0·83 mm. from the anterior end. At its origin it is about 0·02 mm. wide, but it gradually widens to a diameter of about 0·03 mm. at half its length. At this point it sharply increases to a diameter of 0·05 mm. and then goes on slowly widening to attain a maximum diameter posteriorly of about 0·07 mm. The œsophagus is muscular throughout, has no bulb, and no valvular apparatus. There is no trace of an excretory pore, and I was unable to decide with any certainty where the nerve ring crosses.

The intestine is brown in colour and has a wide lumen which becomes globular at the point where it meets the œsophagus. Apart from being displaced by the gonads, it continues backwards uninterruptedly to a point about 0·3 mm. from the anus, where it is suddenly constricted (fig. 15). There follows a section which is much paler in colour and appears to have slightly muscularized walls; this, the prerectum, is about 0·22 mm. long and is rounded posteriorly. From it a narrow

rectum proper extends about 0·08 mm. to open at the anus, about 0·2 mm. from the tip of the tail. The conspicuous anal muscles are just dorsal to and radiate from the anus, and are collected into about six bundles.

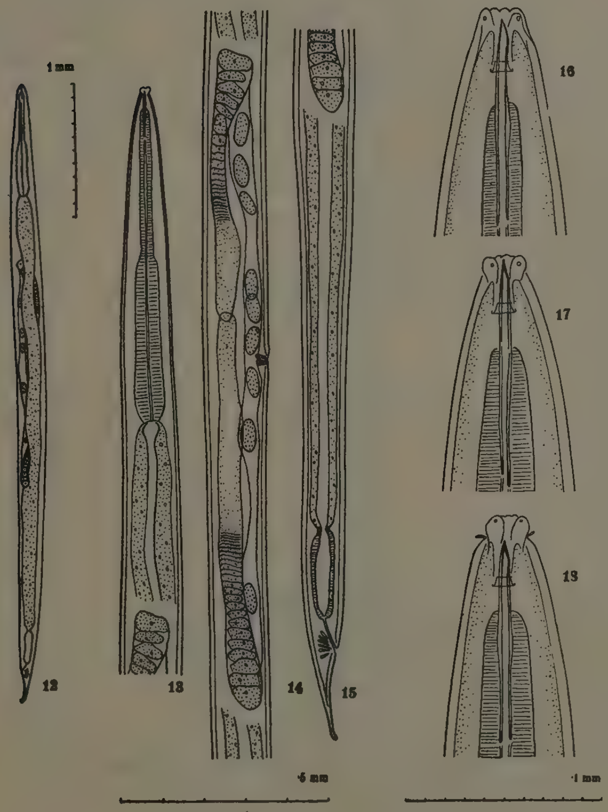
The gonads are paired, opposed and approximately symmetrical (fig. 14). The vulva, situated 1·98 mm. (or 45 per cent. of the body length) from the anterior end, leads into a short muscularized vagina which opens into the long uterine cavity. For convenience this may be spoken of as anterior and posterior uteri, though there is nothing to distinguish them except the position of the vagina. The uteri, then, extend backwards and forwards respectively for about 0·8 mm., the posterior one being usually a little longer than the anterior. At this point they are bent double, approaching one another again, and the reflexed portions are closely packed with developing ova, so that they may be termed ovaries. Further up the ovary the ova become more and more flattened until, in the distal half, cell outlines cannot clearly be distinguished. Terminally, the ovaries usually overlap a little, in the region of the vulva. The uteri contain between them from six to a dozen ova, each ovum measuring, *in situ*, about 0·07 mm. by 0·035 mm. The worms are oviparous.

Discussion.

The literature on this genus is widely scattered, and diagnosis is often a matter of considerable difficulty. I have relied mainly on Micoletzky (1921) who gives an exhaustive key to 72 species of *Dorylaimus* and its subgenera. From this key it became clear that the present species belonged to *Dorylaimus*, *sensu stricto*, and not to any of the other subgenera, but beyond this not one of the species listed had characters that would accord with those of the present species.

To find a species of *Dorylaimus* living in such a medium as sewage is strange, since most members of the genus are soil nematodes abounding near the roots of living plants. In fact, Micoletzky, who treats fully of the ecological aspects of his subject, states that the only species of the genus ever found in a saprobic medium is *D. borborophilus*, found by de Man (1876) in cow dung. The latter species is less than half the length of the present one and has relatively a shorter tail.

Accordingly, I have felt obliged to create a new species for these forms this procedure, if only provisional, seeming preferable to their going unnamed. In view of the habitat's being so atypical for the genus, I have selected the name *saprophilus*.



Dorylaimus saprophilus sp. n., female. Fig. 12, entire worm (lateral view). Figs. 13, 14 and 15, entire worm in three sections (lateral view). Fig. 16, head (ventral view). Figs. 17 and 18, head (lateral view).

Specific Diagnosis.

Dorylaimus saprophilus spec. nov., having the following principal average dimensions. Length 4·38 mm., greatest breadth 0·17 mm., length of œsophagus 0·83 mm., length of spear 0·11 mm., length from anterior end to vulva 1·98 mm., extent of anterior gonad from vulva 0·75 mm., extent of posterior gonad from vulva 0·83 mm., length from anterior end to anus 4·18 mm., length of prerectum 0·22 mm., length of tail 0·2 mm., dimensions of ova 0·07 mm. × 0·035 mm. Having six prominent lips, each with a papilla. The proportion of the narrower part of the œsophagus to the whole being 50 per cent. Proportion of total extent of gonads to total length of body being 36 per cent. Oviparous.

Habitat: in the zooglœal film surrounding the stones in percolating sewage filters, London, 24th July, 1928.

For purposes of easier comparison with other species, and in order to give extreme as well as average measurements, the formulæ of Cobb (simplified) and of de Man are appended here.

De Man Formula.

	Minimum.	*Maximum.*	*Average.*
L	4·05 mm.	4·75 mm.	4·38 mm.
α	21·4	28·5	25·8
β	4·9	5·6	5·3
γ	19·5	28·0	22·8
V	43%	46%	45%

Cobb Formulæ.

Minimum—Maximum.

$$\frac{(2{\cdot}3\text{–}2{\cdot}6)\quad (?)\quad (17{\cdot}8\text{–}20{\cdot}4)\quad (43{\cdot}0\text{–}46{\cdot}0)\quad (95{\cdot}0\text{–}96{\cdot}0)}{(1{\cdot}4\text{–}1{\cdot}6)\quad (?)\quad (3{\cdot}2\text{–}3{\cdot}8)\quad (3{\cdot}5\text{–}4{\cdot}5)\quad (1{\cdot}4\text{–}1{\cdot}55)}\quad (4{\cdot}05\text{–}4{\cdot}75\text{ mm.}).$$

Average.

$$\frac{2{\cdot}43\quad (?)\quad 18{\cdot}9\quad 45{\cdot}0\quad 95{\cdot}3}{1{\cdot}51\quad (?)\quad 3{\cdot}43\quad 3{\cdot}91\quad 1{\cdot}48}\quad 4{\cdot}38\text{ mm.}$$

Rhabditis species.

Several specimens of a form belonging to the genus *Rhabditis* were discovered, but unfortunately only a single male was forthcoming, and

he had lost his tail in some way and was trailing a formless stump behind him (fig. 22). It was impossible to be certain whether all the bursal rays were intact or whether one pair had been carried away with the tail. This doubt, and the unknown length of the tail, made diagnosis rather hazardous. Such as it is, the material does not definitely corre-

Rhabditis species. Fig. 19, female (lateral view). Fig. 20, male (ventral view). Fig. 21, anterior end of female (lateral view). Fig. 22, posterior end of male (ventral view).

spond to any of the 55 species listed by Micoletzky (1921), but I hesitate to erect a new species on such scanty foundations. A brief account of the more interesting features will, however, be given.

The female worm is about 1·86 mm. long, the single male was 1·18 mm. long to the tip of the bursa. The greatest breadths are about 0·07 mm. (female) and 0·05 mm. (male) respectively. The principal ratios, giving values for the female first, are: α 27, 23; β 6·2, 4·8; γ 19·5 (male unknown), ; and V 52%. There are six vaguely distinguishable lips and a cylindrical buccal capsule of between $\frac{1}{6}$ and $\frac{1}{7}$ the total length of the œsophagus. The anterior bulb is fairly well marked, and the nerve ring crosses just behind it. The posterior bulb is of the usual type and contains a valvular apparatus. Immediately opposite the anterior end of the valve a fine excretory pore is distinguishable, on the ventral side, in the female. In the intestine the cells of the wall show up clearly. There is a pair of small anal glands in the female. The female gonads are paired, opposed, reflexed and symmetrical. The worm is oviparous. Posterior to the valvular bulb, and lying dorsally, there was in one female a vague suggestion of a three-celled gland. I have figured this (fig. 21), but as it was not definitely seen in the male or in the other females its existence as a gland is open to doubt. It may possibly have been a deformity of the intestinal wall.

In the male the gonad (fig. 22) is of the usual single, reflexed type; there is a distinct vesicula seminalis posteriorly, separated from the testicular tube by a constriction (fig. 20) and, near the anus, this appears to give off a diverticulum directed anteriorly (fig. 22). Two elongated, lateral anal glands extend forwards for a distance of 0·15 mm. from the anus. The spicules are of characteristic shape, knobbed anteriorly, and are 0·062 mm. long. The bursa is well developed and originates anteriorly at the level of the spicule-knobs. Two short, stumpy bursal rays are definitely pre-anal in position and are separated from the others by a wide space. There follows a group of four long, slender rays ad-anal in position. The next (seventh from before backwards) is considerably stouter and is attached to the edge of the bursa at its tip, in such a way as to retract the membrane a little at this point. There are certainly two more rays posterior to the seventh, these being

long and slender like those of the ad-anal group. The seventh, eighth and ninth rays are decidedly post-anal. All the rays bear small knobs terminally, and all are ventro-lateral in position. In the formula devised by Örley and used by Micoletzky, in which the rays are detailed from behind forwards, the arrangement in this species would be as follows: $(1-2)\ 3\ (4-7) + (8-9)$, the plus sign marking off the rays of the pre-anal group.

References.

Bütschli, O., 1876.—"Untersuchungen über freilebende Nematoden und die Gattung Chætonotus." *Z. wiss. Zool.*, xxvi, 363-413. (W.L. 23635.)

Cobb, N. A., 1893.—"Nematodes, mostly Australian and Fijian." *Macleay Mem. Vol., Linn. Soc. N.S.W.*, 252-308.

— 1913.—"New Nematode Genera found inhabiting Fresh Water and Non-brackish Soils." *J. Wash. Acad. Sci.*, iii, 432-444. (W.L. 11600.)

Man, J. G. de, 1876.—"Onderzoekingen over vrij in de Aarde levende Nematoden." *Tijdschr. ned. dierk. Ver.* (2); Not Consulted. (W.L. 21275.)

— 1896.—"Description of Three Species of Anguillulidæ, Observed in Diseased Pseudo-bulbs of Tropical Orchids." *Proc. Lpool. Biol. Soc.*, ix, 76-94. (W.L. 16792.)

Maupas, E., 1900.—"Modes et Formes de Reproduction des Nématodes." *Arch. Zool. exp. gén.*, 3rd Series, viii, 463-624. (W.L. 1915.)

Micoletzky, H., 1921.—"Die freilebenden Erd-Nematoden." *Arch. Naturgesch.*, lxxxvii, Abt. A, 1-650. (W.L. 1782.)

Peters, B. G., 1928.—"Some Nematodes of a Sewage disposal Plant," an otherwise unpublished section of a Thesis, "*Saprophytic Eelworms*," submitted to the University of London, 20 pp.

— 1929.—"A New Wedge-colorimeter used in a Biological Investigation of Sewage." *J. Helm.*, vii., 201-214.

— 1930.—"A Biological Investigation of Sewage." *J. Helm.*, viii, pp. 133-164.

SCHNEIDER, A., 1866.—" Monographie der Nematoden." VIII + 357, 28 plates. (Berlin.)

STEINER, G., 1914.—" Freilebende Nematoden aus der Schweiz." *Arch. Hydrobiol. Plankt,* IX ; Not Consulted. (W.L. 1769.)

ZIMMERMANN, A., 1898.—" De Nematoden der Koffiewortels." *Meded. PlTuin Batavia,* XXVII ; Not Consulted. (W.L. 12949.)

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp.* 185-196.

Observations on the Life-Cycle of *Heterodera schachtii*.

By MARJORIE J. TRIFFITT, M.Sc.

(*Field Officer, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

INTRODUCTION.

THE early accounts of the morphology and life-cycle of *Heterodera schachtii*, were, like much of the subsequent work on this subject, the result of observations carried out on the strain of *H. schachtii* parasitic on sugar-beet in Germany.

Much of the research work done by 19th and early 20th century workers has remained unconfirmed, and certain recent observations have indicated possible errors in their conclusions which have hitherto escaped detection. These conclusions have been generally accepted, and assumed to be relevant to strains specialised on hosts other than the sugar-beet.

The present writer has investigated certain aspects of the life-history of two strains of the parasite occurring in Britain, and observations which contribute to a better understanding of the life-cycle of these strains are embodied in this publication.

HISTORICAL.

Before detailing the experiments and observations on which the new conclusions are based, some account of the observations of other workers must be given.

Strubell (1888), published a more detailed account of the morphology of the nematode than has been produced by any subsequent writer. Included in this morphological study, however, are some remarks on the life-cycle which have been proved inapplicable to three of the known British strains.

Following an account of the formation of the brown cyst, Strubell noted that the development of the female worm occupies from 4 to 5 weeks. He then states that therefore in a single season, as many as six or seven generations of this nematode may be produced.

Chatin 1887 and 1888 carried out a more detailed study of the later developmental stages of the female with special reference to the changes taking place in the body wall, leading to the production of the brown cyst. With regard to these changes, Chatin points out that the fate of the " epiderm " varies according to certain conditions. If the eggs are immediately to be set free it ruptures and disintegrates, but if, on the other hand, the eggs are to remain in the brown cyst, the latter is formed by the covering and binding together of the integument by an exudation formed by the " endoderm " previous to its disintegration.

The more recent researches of Fuchs (1911) confirm Chatin's observation that the formation of a brown cyst does not necessarily occur to complete the development of every female nematode. According to this author, although brown cysts are formed throughout the summer in small quantities, the majority of the females developed during the warmer periods of the year do not assume the brown stage, but fall from the roots of the host plant when still white, and allow the larvæ to escape and infect fresh roots at once. Only on the approach of cold weather are brown cysts formed in any quantity, and then they represent by far the greater proportion of the output of mature females, the few which do not undergo this change perishing with their contents during the winter. Fuchs was able to demonstrate effectively by experimental methods, that without the protection of the brown cysts the eggs and larvæ of the parasite are destroyed by winter conditions.

Experiments to demonstrate the percentage production of brown cysts throughout the summer and autumn were also carried out by Fuchs. These showed that within a specified period seven per cent. of all females developed in August assumed the characteristics of the brown cyst, nine per cent. in September, thirty-three per cent. in October and sixty-six per cent. in November. The nature of these experiments was not, however, in the present writer's opinion, such as to provide conclusive proof that these percentages give a true idea of the relative numbers of brown cysts formed, as the counts were made on one occasion only, and the females found then to be in the white stage were not subsequently examined.

Sengbusch (1927), also using a strain of *H. schachtii* parasitic on the sugar-beet, found that one hundred per cent. of the white female nematodes, formed even as early in the year as May, became brown cysts

when kept in sand, soil or water, either at room temperature or at a temperature of only 1° to 2° C. He therefore regards as a fallacy the assumption of Chatin and Fuchs, that only a proportion of the females, varying according to the season of their development, are capable of developing into the brown cyst stage.

Sengbusch does not, however, record any observation on the number of generations which the nematode is capable of producing in a single season.

Duffield (1927), studying *Heterodera schachtii* parasitic on Hops in Britain, found that only a single generation of the nematode attacked this host during the year. This conclusion was arrived at after a prolonged study of the development of the females and their contained eggs.

During his researches on the bionomics of *H. schachtii*, Fuchs found that under suitable conditions the escape of the larvæ from the cysts might commence within a few weeks of the formation of the latter ; he therefore investigated the period during which cysts containing viable eggs remained in the soil, and attempted to determine the factors governing the escape of the larvæ. Cysts were isolated from infected land which had remained fallow and free from weeds for five, four and three years respectively. These were dissected and the eggs and larvæ within them were estimated with the following results. Of the five year old cysts, fifty-five per cent. were empty, and of the remainder one only contained more than 50 eggs. Of the four year old cysts twenty per cent. were empty and 112 eggs was found to be the maximum number, while in the three year old cysts 160 eggs was the maximum number, the average count being 40 to 50.

From these figures and the results of further experiments on the factors governing the emergence of the larvæ from the cysts, Fuchs concluded that the larvæ emerge as they mature and as temperature and moisture conditions are favourable, and that the presence of the host plant is not a factor of any consequence in causing the escape of the larvæ.

Observations on the formation of Brown Cysts.

During the course of experiments on strains of *H. schachtii* parasitic on potatoes in this country, the present author has made various observations on the formation of brown cysts. These confirm Sengbusch's

remarks and also reveal an interesting seasonal variation in the time factor, for which no explanation is at present apparent.

Throughout the spring, summer and autumn of the past three years, numerous series of pot experiments have been performed, in which counts have been made of the female nematodes visible on the roots of the plants. This was done as a means of estimating the intensity of the infections. The counts were made by removing the containing pots and thus exposing the mass of roots and soil ; the majority of the roots were then visible and the globular white females, which tended to congregate on the outer surface of the roots, possibly on account of the greater aeration to be obtained in close proximity to the porous pot, were plainly visible and could easily be counted. Counting, however, became very difficult when sufficient time was allowed to elapse for the females to undergo transformation into the brown cyst stage, as the latter were easily overlooked against the dark colouration of the soil. Under these conditions the margin of error was judged to be so greatly increased as to render the counts useless, and great care was accordingly taken to avoid this occurrence.

It was found that, during the early period of the year, the plants might safely be allowed to grow for eight or nine weeks without fear of any of the females becoming brown cysts. Towards the end of the season, however, not more than seven weeks could be allowed, and even then a small number of brown cysts were occasionally present on the roots. Further, from mid-August onwards, even after a growing period of only six weeks, when the roots bearing the nematodes were exposed to the air for twenty-four hours, all the females visible upon them, irrespective of size, were found, during that period, to have assumed the brown colouration and become cysts. Earlier in the season, in May and June, although this change took place in about fifty per cent. of the females within twenty-four hours, the remainder changed colour more slowly, some remaining apparently unchanged for over a week, so that from seven to twenty days was necessary for the complete transformation of all the females on a single plant into brown cysts. Nevertheless, it was found that, under these experimental conditions, every female nematode which had attained sufficient size to be distinguishable without the aid of a lens, ultimately, upon exposure to air, attained the characteristics of the brown cyst. Miles (1930) points out that the destruction

of plants by autumn frosts before all the nematodes have completed their development accounts for the occurrence of pyriform or flask-shaped cysts with their contents at various stages of development, frequently found in soil during the winter.

These observations, agreeing with Sengbusch's experimental results, led to further investigations on cyst formation in a strain attacking Mangolds in Britain. The latter, a comparatively omnivorous strain occurring in the Bristol area, was discovered by Mr. L. N. Staniland and Dr. C. L. Walton and has already been the subject of a morphological study by the present writer, with which were included certain field observations dealing with the white stage females. In this publication it was noted that, towards the end of October, numerous white stage females were still adherent to the roots of the infested plants, and, as the land was ploughed, white forms in the soil were brought to the surface where they remained visible as such for two days during which observations were carried out.

This strain of *Heterodera schachtii* has been successfully established in a field plot at Winches Farm where further investigations are being made. Mangolds grown in this plot in 1929 showed a fairly heavy infection, and in late October when the crop was lifted numerous white stage females were still present on the roots. Brown cysts were also present, but apparently in comparatively small numbers. No attempt was made to estimate the relative proportions of brown and white forms, as it was thought that a large proportion of the former had probably already become detached from the roots.

Examinations of the soil after the removal of the crop showed that white forms persisted though in decreasing numbers, for a period of almost two weeks, after which time they had completely disappeared. It remained unknown whether their disappearance was due to their progressive development into brown cysts or their destruction by weather conditions.

During the 1930 season, Mangolds were again grown on this infected land, and white females of approximately the maximum proportions recorded for the brown cysts of this strain were present on the roots in mid-August, although no brown cysts could be found. Immature females, were also abundant. To determine the fate of these forms a number of infected plants were removed from the soil, half of their

number were left with their roots exposed to the air at room temperature, and from the other half those portions of roots bearing nematodes were removed, and placed in a petri-dish containing water which had been run through a pot of soil in which Mangolds were growing. These were also kept at room temperature. Daily examinations showed that the white forms in water retained their normal outline and gradually assumed a brown colouration, the larger individuals within two to four days, smaller forms more slowly, a fortnight being required in some instances for the transformation to be completed. In the nematodes exposed to air a certain amount of shrivelling of the body wall was noted, especially among the smaller individuals, and the change to the brown cyst stage took place much more slowly than in the previous instance. Nevertheless, again every visible nematode ultimately acquired the characteristics of the brown cyst stage. Similar observations carried out in October gave the same result, although as had already been noted in the potato strain, the changes were effected more rapidly at this time than they had been earlier in the year.

Liberation of the Larvæ from newly formed Cysts.

As all the females of two distinct strains of *H. schachtii* were thus proved to be potential brown cysts, the statements of early workers that larvæ hatch and are liberated early in the season from certain females incapable of assuming the resistant brown form, required investigation. This more especially since these early-hatching larvæ were supposed to produce a second generation of mature white forms which repeated the reproductive process until as many as six or seven generations were produced in a single season.

Short lengths of roots bearing in all 100 large white females of the potato strain were removed in May from potato plants which had been grown for two months in infected soil. Ten per cent. of these females were examined and found to contain unsegmented eggs only, except in a single individual in which a few eggs had reached the early stages of segmentation. These portions of root were placed in water containing potato root excretion and incubated at 25° C. After five days a small proportion of the nematodes showed signs of browning—the epiderm being of a light yellowish shade. Five of these yellowish females and five which appeared unaltered were dissected. In all cases a few eggs

had reached the early segmentation stages of development, the remainder being still undifferentiated. Three days later half of the remaining nematodes had become brown cysts. Five of these cysts and five white forms were dissected; the latter contained undifferentiated eggs and eggs in the early stages of segmentation, but advanced segmentation stages were recovered from the brown cysts although no embryonated eggs were yet present. After a further period of four days, only sixteen white forms remained, and dissections showed that over fifty per cent. of the eggs in these, as also in the brown forms, were embryonated. Three days later no white forms remained. The cysts were kept in fresh root excretions at 25° C. and daily examinations were made for escaped larvæ. Twenty-nine days after the removal of roots from the growing plants five larvæ escaped from the brown cysts. Larvæ continued to be liberated in increasing numbers during the following month, after which the observations were abandoned.

Similar observations carried out in August on the strain attacking Mangolds also showed that no larvæ were liberated until the brown cyst stage had been attained, and that a period of about a month elapsed between the removal of the female nematodes from the plants and the escape of larvæ from the resultant cysts.

Number of Generations occurring in One Season.

Although larvæ could thus be hatched from newly developed cysts by laboratory methods it was by no means certain that early hatching would take place in the soil, where the formation of brown cysts was known to be much more slowly accomplished. In order to determine whether more than one generation of the nematode could occur in a single season without the exposure necessary to hasten the production of the brown cysts, the following experiment was set up.

Two potatoes were planted in infected soil on March 1st and grown in a cool greenhouse. Periodic examinations of the roots showed that the development of the nematode was very slow, due no doubt to the dormancy period. On May 23rd however, large white females which were judged to have attained their maximum proportions were fairly abundant. The plants were then removed from the infected soil and their roots very carefully washed to remove earth which might contain old cysts or larvæ. One shoot was left on each plant to avoid the imme-

diate destruction of the host, and the plants were repotted in clean soil in a single large pot into which a sprouted tuber was also introduced. The new tuber grew well and the old plants gradually died down. Examinations of the roots of the new plant were made in July and early August, but no traces of nematode attack could be detected. On August 6th, as the shoots of this plant showed signs of dying down another tuber was introduced into the same soil. No traces of eelworm attack were found on this last plant until September 23rd when a single immature female was found and a Baermann extraction of the roots produced one mature and two immature males.

Thus in a period of almost seven months, and under conditions more favourable for re-infection than would obtain under field conditions, only two generations were produced. Miles (1930), recording field observations, states that new cysts containing active larvæ were not found until July 23rd, but that after that date it was impossible to determine whether first stage larvæ in the root tissues had escaped from newly formed or over-wintered cysts. He also points out that cysts may continue to develop as late as October on stolons and tubers. These observations, together with the results of the experiment described above, point to the conclusion that, under normal conditions of potato culture as practised in this country, only a single generation of the nematode would come to maturity each season, although under exceptional conditions the possibility of a second generation occurring is not excluded.

A similar experiment could not be carried out with the strain attacking mangolds, as the dormancy period in this strain is more prolonged than in the potato strain. Mangold seed was sown in pots of infected soil in January and germinated under greenhouse conditions, but these plants failed in every case to show infection before mid-June and the material obtained from them in July was too scanty to carry on the experiment. Abundant quantities of white females attached to roots from the field crop were, however, potted with mangold seedlings in September. After eight weeks the roots of these seedlings were examined and failed to show any evidence of infection ; at the end of November, however, twelve weeks after the experiment was set up, a few immature white females were found to be present. It is therefore concluded that in this strain, as in hop and potato strains, only one generation of nematodes is normally produced annually.

Successive Infections of Host-plants in Infected Soil.

As laboratory experiments have shown that larvæ are, under suitable conditions, liberated gradually over a long period from cysts isolated from soil, it seemed possible that the production of successive infections from old cysts was responsible for the assumption that several generations were produced yearly.

Successive potato plants were therefore grown for periods of six to nine weeks in pots of infected soil throughout the 1930 season. The intensity of infection as indicated by the number of white stage females was roughly estimated, and the infected roots were removed from the soil on each occasion before replanting. Infections occurring every month from May to September were obtained from the two pots of soil in use. The counts of the second infections obtained in June and July respectively, greatly exceeded the counts of the first infections taken in mid- and late- May, while the third counts were even lower than the first. This result corresponds with those of the laboratory hatching experiments previously recorded, and may be considered to represent the effects of the natural hatching rhythm taken in conjunction with the gradual exhaustion of the cyst-contents by the liberation of successive batches of larvæ.

The Influence of Root Excretions of the Host Plant on the Hatching of the Larvæ.

As stated above, Fuchs concluded that the presence of the host plant did not materially affect the rate at which larvæ emerged from the cysts, but that under suitable conditions of temperature and moisture a proportion of the eggs, viz., those which had reached maturity, were liberated each season, over a period of years. Rensch (1924), found that larvæ emerged from cysts of a beet strain in considerable numbers during a short period only, when kept in water at 25° C. without any excretions from the host plant, but that when the number of escaping larvæ became very small under these conditions, it could be immediately increased by the addition of substances obtained from the host plant to the containing water.

Hatching experiments with the British potato strain have given strikingly different results. Repeated experiments have shown that,

without the presence of root excretions, only a very small number of larvæ can be induced to emerge from the cysts under otherwise favourable conditions, whereas when even a trace of the root excretions of the potato plant is present, the larvæ escape from the cysts in considerable numbers. By renewal of the root excretions a high rate of hatching may be maintained over a fairly long period, after which the number falls although a few larvæ continue to escape for a further period varying in duration with different cyst samples.

To test Fuchs's conclusion that the number of larvæ that escape is dependent on the number of eggs that reach maturity each season, the following experiment was performed. Fifty cysts were taken from the dried roots of potato plants grown the previous year and stored during the winter. As the eggs contained in these cysts had been given no opportunity of hatching and cysts of approximately equal size had been chosen it was assumed that each contained about three hundred eggs. These cysts were placed in an incubator at 25° C., in water containing potato root excretions on May 9th. The medium was renewed at frequent intervals until October 29th when the cysts were carefully dissected and the unhatched eggs and free larvæ which they contained were counted. Twelve of the cysts were then found to contain no larvæ or unhatched eggs, nineteen contained less than 5, ten between 5 and 10, four between 10 and 20, four between 20 and 50, and one 84 unhatched eggs and larvæ. The total number of unhatched eggs and larvæ in the fifty cysts dissected was 362, giving an average of 7·2 per cyst. It should be noted, however, that all the free larvæ were dead, the majority of the unhatched eggs were not embryonated but contained granular vacuolated contents only, and in the cysts where high egg counts were made, the eggs were deep brown or black in colour, apparently due to attack by some fungus or bacterium. Although it was impossible to determine with accuracy the number of eggs which had remained viable, it was roughly estimated that these did not exceed ten per cent. of the total number.

It is therefore concluded that approximately all the eggs contained by overwintered cysts of the potato strain are capable of hatching during the first season following their development under favourable physical conditions and in the presence of the stimulus exerted by the host plant.

Conclusions.

From the results of the experiments described above it is concluded that every female worm which attains sufficient size to be easily detected by the naked eye before the destruction of the host plant takes place, is capable of undergoing transformation into the resistant brown cyst stage. Whether this consistently occurs under field conditions at the end of the growing season remains unknown, but from Sengbusch's observation that 100 per cent. of white females become cysts at temperatures of 1 to 2° C., it may be assumed that only a very small percentage of immature females developed late in the season will suffer destruction by weather conditions before the resistant characters can be attained. The factors influencing the rapidity of the transformation at different periods of the year remain to be investigated, as do those governing the dormancy period.

The production of six or seven generations of nematode in one season on a single crop of host plants is proved to be a fallacy at least as regards the British potato strain and the less specialised strain attacking mangolds. The possibility of two generations occurring under very exceptional conditions, is not, however, totally excluded. This is of particular importance in regard to the multiplication and dissemination of the parasite. Experiments by Fuchs show that the larvæ of *H. schachtii* may travel from three to four metres by their own activity in search of food. Fuchs therefore concludes that with five or six generations a distance of fifteen to twenty-four metres may be covered by the parasite in one season. With only a single generation involved the annual spread of infection from any given point becomes comparatively small, apart from mechical transmission.

Although only one generation of the nematode is produced, successive crops of the host plant grown throughout the summer in infected soil will be attacked by larvæ liberated from the cysts of previous seasons. This fact, together with the seasonal variation in the time necessary for the transformation of the white form into the brown cyst, has probably been responsible for the persistent assumption that several generations of the nematode complete their development every year.

Finally, it has been shown that the larvæ of the potato strain do not hatch in any considerable number except in the presence of the root

excretion products of the host plant. When, however, physical conditions are favourable and an abundance of root excretion is available for as long a period as six months, the emergence of the larvæ continues until by the end of the period the cyst contents are practically exhausted.

It is of possible significance to note that although the larvæ of Fuch's strain hatched freely from the cyst in the absence of the host plant, after a five years fallow a considerable number of eggs still remained in some of the cysts isolated from infected soil. Since the larvæ of the potato strain do not become abundantly liberated in the absence of the host plant the result of a five years fallow might be expected to be even less satisfactory in ridding the soil of the eelworm pest than occurred in Fuch's experiment with the beet strain.

References.

Chatin, J., 1887.—" Sur les Kystes bruns de l'anguillule de la betterave," *C. R. Acad. Sci. Paris*, vol. cv, pp. 130-132. (*W.L.* 6628).

— 1888.—" Sur la structure des téguments de l'*Heterodera schachtii* et sur les modifications qu'ils présentent chez les femelles fécondées," *C. R. Acad. Sci., Paris*, vol. cvii, pp. 139-141. (*W.L.* 6628).

Duffield, C. A. W., 1927.—" The beet eelworm (*Heterodera schachtii* (Schmidt)) : Its life history when found on hops in this country," *J. Southeast Agric. Coll. Wye, Kent*, No. 24, pp. 56-58.

Fuchs, O., 1911.—" Beiträg zur Biologie der Rubennematoden *Heterodera schachtii*," *Z. landw. Vchsw. Ost.*, Jahrg. 14, pp. 923-949. (*W.L.* 23488).

Miles, H. W., 1930.—" Field Studies on *Heterodera schachtii* Schmidt in relation to the pathological condition known as " potato sickness," *J. Helm.*, vol. viii, pp. 103-122.

Rensch, B., 1924.—" Eine neue Methode zur Bekämpfung der Rübennematoden," *Mitt. deuts. Landw. Ges.*, Bd. 39, pp. 412-414. (*W.L.* 13900).

Sengbusch, R. v., 1927.—" Beiträg zur Biologie des Rubennematoden *Heterodera schachtii*," *Z. PflKrank.*, vol. xxxvii, pp. 86-102. (*W.L.* 23540a).

Strubell, A., 1888.—" Untersuchungen über den Bau und die Entwicklung des Rubennematoden '*Heterodera schachtii*, Schmidt,' " *Bibl. zool.* (Cassel), Heft. 2, pp. 1-49.

Triffitt, M. J., 1929.—" Further observations on the morphology of *Heterodera schachtii*, with remarks on the bionomics of a strain attacking mangolds in Britain," *J. Helm.*, vol. vii., pp. 119-140.

— 1930.—" On the bionomics of *Heterodera schachtii* on Potatoes, with special reference to the influence of mustard on the escape of the larvæ from the cysts," *J. Helm.*, Vol. viii, pp. 19-48.

Journal of Helminthology, Vol. VIII, *No.* 4, *December,* 1930. *pp.* 197-210.

On *Tylenchus agrostis* (Steinbuch 1799).

By T. Goodey, D.Sc.

(*Principal Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine*).

Introduction.

Galls, in the inflorescences of various species of grasses, caused by nematodes have been known since Steinbuch first described them, in 1799, in the flowers of *Agrostis capillaris* and *Phalaris phleoides*. Since that time they have been found in several other species of grasses and have been studied principally by entomologists interested in galls caused by insects. The causative organisms have received but little serious attention from helminthologists in recent years.

The question of the specific identity of *Tylenchus agrostis* and *T. phalaridis* has remained open since 1799 when Steinbuch named them along with *T. tritici*. No one has been able to say whether they were identical with or distinct from the last-mentioned species.

Experiments have been made by Marcinowski in Germany and by Bessey and Leukel in U.S.A. to test whether *T. tritici*, causing galls in wheat, will produce them in various grasses and, vice versa, whether the worms responsible for them in grasses will give rise to them in wheat, but with entirely negative results. There has thus been reasonable experimental ground for the assumption that the species are distinct. So far, however, no one seems to have compared the adult worms from their respective galls.

The writer has recently been able to pursue some investigations on the nematode galls in the panicles of *Agrostis stolonifera* and to make a detailed comparison of the adult worms with those of *T. tritici* from wheat with the result that there seem to be sound reasons for considering *Tylenchus agrostis* as a species distinct from *T. tritici*.

Historical.

Steinbuch (1799), described how, in a wood in the vicinity of Erlangen, he collected specimens of a grass, belonging to the genus *Agrostis*, in the inflorescences of which he found certain flowers with greatly elongated glumes whilst others were small and of normal size. He discussed at length the specific identity of the grass and finally referred it to *Agrostis capillaris*. In the flowers with long glumes and paleæ he found, instead of the reproductive organs, only a long, shuttle-shaped, purple gall. It was soft, had a delicate covering wall and the contents, when pressed out and examined in a drop of water, under the microscope, were found to consist of a mass of minute worms of different sizes as well as eggs containing coiled embryos. He described and figured these organisms and recognized the adult females containing an ovary though he mistook the vulva for the mouth and thought the eggs were passed out through the mouth. He did not distinguish the male worms, though it is clear from his drawings, that these were present. He allowed the larvæ to become dry on the glass slide and found that they revived on remoistening, though the large forms failed to do so. He classified the worms as belonging to the genus *Vibrio* of Müller and named then *V. agrostis*, distinguishing them from the worms causing galls in wheat, of which an account had appeared a few years previously by Roffredi, and to which, in this paper, for the first time he applied the name *Vibrio tritici*, apparently in a strict bi-nominal manner.

Steinbuch's name *V. tritici* was quite overlooked by Bauer (1823) who again used the same name for the worms from wheat galls without any mention of Steinbuch.

In the same paper Steinbuch described another species of worm, viz., *V. phalaridis*, obtained from galls in the flowers of another grass, *Phalaris phleoides*, now called *Phleum Böhmeri*. It is very doubtful whether this is a distinct species from *T. agrostis*, and the question is discussed at the end of the present paper.

Ehrenberg (1838) transferred both *V. agrostis* and *V. tritici* to his genus *Anguillula*, whilst Dujardin (1845) placed *V. tritici* in his genus Rhabditis. Diesing (1851) put both *tritici* and *agrostis*, which he misnamed *graminis*, in his omnibus species *Anguillula graminearum*.

Bastian (1865) placed both *tritici* and *agrostis* in his genus Tylenchus, though he misnamed the grass form *agrostidis* for *agrostis*, as Stiles and Hassall (1920) point out. Most subsequent writers on grass galls have followed Bastian in this error. He wrote as follows concerning it. "It seems to me most probable that this species is distinct from *Tylenchus tritici*; the determination of this question, however, must be left to future observers."

Bessey (1905), writing on nematode galls in grasses, mentioned that the glumes of affected flowers are much enlarged. He also recorded galls from certain species not previously known to be attacked by nematodes. Marcinowski (1909) gave a brief account of the points of difference enumerated by Steinbuch between *T. agrostis* and *T. tritici* to none of which, she considered, could much weight be given. She emphasised the close relationship of the two species and briefly described some of the symptoms of attack, but failed to mention the elongation of the glumes and paleæ. She also gave a list of grass species on which galls attributed to nematodes have been recorded.

Houard (1908-13), in his great work on plant galls, gives brief descriptions of nematode galls on different species of grasses and the principal pathological results. He adds a full bibliography.

Material.

The material used in the present investigation consisted of galled flower spikes of *Agrostis stolonifera* and *A. tenius* sent in July, 1929, from the Official Seed Testing Station, Cambridge, by Mr. R. A. Finlayson, to whom the writer's thanks are due.

In July, 1930, galled inflorescences of *Agrostis stolonifera* and *A. vulgaris* were received from Mr. J. Staley, of the British Mosquito Control Institute, Hayling Island, who had found and collected them on the island. The writer desires to express his thanks to Mr. Staley for this supply of material which was received in a fresh condition.

A quantity of dried galls from the Cambridge material furnished a source of infection for an experiment set up in March, 1930. Three six-inch flower pots were filled with partially sterilised soil and were sown thinly with seed of *Agrostis stolonifera*. In two of the three pots dried galls were scattered amongst the seed whilst the third was left without galls to serve as control. The pots were kept in a greenhouse

until the seedlings were two to three inches high, when they were taken out of doors, sunk in ashes and covered with a wire cage to protect from birds. Growth continued throughout the spring and summer and infected panicles appeared in good numbers towards the end of September and throughout October. From these it has been possible to study still further the effects of parasitism on the plant and to make detailed observations on the anatomy of the adult worms and their development within the galls.

Effect on the Host.

So far as the writer's observations go, affected grass plants show no marked twisting or curling of the leaves such as that exhibited by young wheat plants infected with larvæ of *T. tritici*. The two infected pots revealed no apparent difference, to the naked eye, from the control pot until the appearance of the panicles which appeared much earlier in them than in the control. A well galled panicle may be stunted in growth and held within its ensheathing leaves ; a condition which was noted by Steinbuch. The leaf blades on some of the affected flower stalks in the two pots also seemed to be shorter and broader than those of normal flower spikes. The uppermost blade was only about half an inch long and the lower ones from one and a half to two inches long, whereas on normal spikes the leaf blades may be three to four inches long.

The normal panicle is loose and has a light and elegant, open appearance. This is frequently the case also with affected panicles, but in some cases the nematode attack results in the panicle having a very compact appearance, due to the shortening of the main axis of the inflorescence and of its numerous lateral branches which carry the flowers. (*Vide* plate.)

A panicle may show normal and galled flowers, the latter being very noticeable because of their much greater length. (*Vide* plate.) Figs. 1 to 4 show the various parts of a normal flower and a galled one from *Agrostis stolonifera* enlarged to the same scale. A normal flower is made up of two glumes, an outer and an inner, with the base of the former wrapping round that of the latter. The outer glume is about 2 mm. long, whilst the inner is a little shorter. Within these are two paleæ, outer and inner, the base of the former again enclosing that of the latter. They are thin membranous structures with rather blunt tips. At their base

and in the front of the flower are two small leaf-like scales, the lodicules. The ovary is closely invested by the inner palea and carries two feathery stigmas. In addition, there are three stamens, each having a slender stalk arising from the base of the flower and carrying a large anther.

In the parasitized flower the glumes are greatly elongated and may be two or three times as long as the normal ones. They closely envelop the outer palea which is often from five to 8 times the length of the normal one and protrudes from the glumes like a stout bristle. It is usually tightly rolled longitudinally and entirely encloses the colourless thin inner palea. This also is frequently much longer than the normal one, but is very variable in length. Within it lies the cigar-shaped purple gall, the tip of which is whitish in colour and the base somewhat brownish green in tint. It is, presumably, a much modified ovary.

There is complete suppression of the lodicules and stamens. The above description applies to the galled flowers most commonly found, but there is greater suppression of the outer flower parts in some cases, particularly on panicles of entirely galled flowers. In one of these, the individual flowers of which were dissected, there was much variation in the amount of reduction, but most often the inner palea was entirely lacking, whilst the outer one was very long. In many flowers both paleæ were missing, the gall being enclosed between the two elongated glumes. Occasionally, also, only one long glume was present, somewhat rolled longitudinally and surrounding the gall.

By dissecting young parasitized panicles removed from the enclosing leaf-sheath, young stages of the galls were found as short round protuberances within the lengthened glumes and paleæ. The apex of each usually showed a sort of cleft or depression as though the gall were formed from two rudimentary leaf scales, the anterior free edges of which came together to form the cleft. By gentle pressure on the surface of some of these young galls, larval nematodes were forced out at the anterior cleft. By dissecting others, various developmental stages of the parasite were obtained.

The Parasite.

As a rule there is only one adult worm of each sex per gall, but occasionally one finds one male and two or three females, and still less frequently, two males and two or three females. In one gall a solitary male was found.

The adults are relatively stout worms, and the male is distinctly smaller than the female, which often has a rather coarse granular appearance, due to the dark intestine and the reproductive organs. The female also has a habit of coiling watch-spring-wise, and always assumes this position when killed by heat. The male, on the other hand, remains almost straight or but slightly bent into an arc.

The females lay an abundance of eggs, which give rise to first stage larvæ and these, after undergoing a moult, become second stage larvæ. The latter occur in abundance in ripe galls. The parent worms become moribund, but do not disintegrate entirely, however, since one can nearly always obtain them along with motile larvæ by soaking dried galls in water.

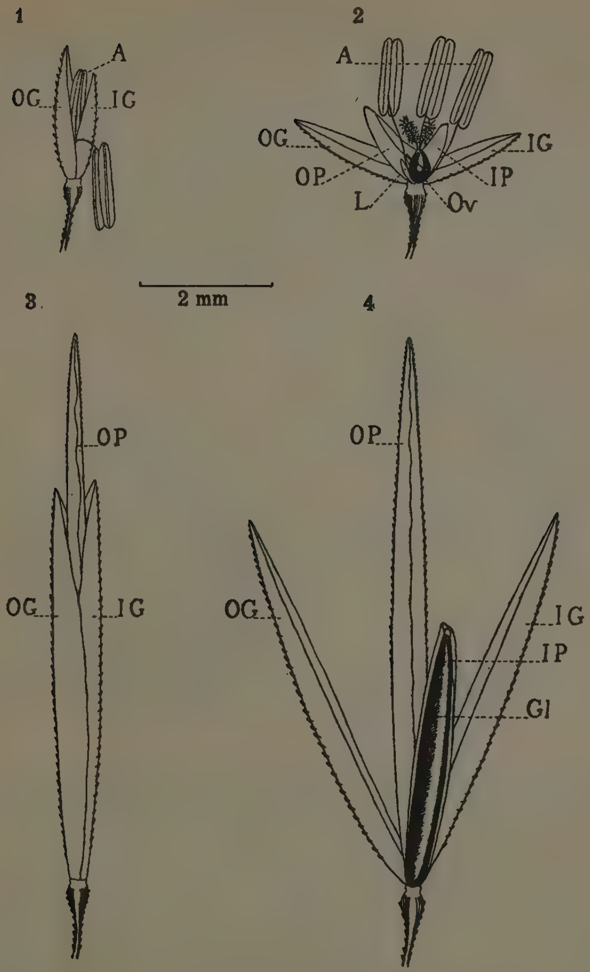
The ripe galls fall to the ground and the wall becoming moist and soft the second stage larvæ are able to make their way out and infect grass seedlings of the next generation.

Morphology.

Female. Principal measurements :—Length, 1·5 mm. to 2·7 mm. ; greatest breadth, 0·09 mm. to 0·14 mm. ; length of œsophagus, 0·18 mm. to 0·23 mm., vulva to tip of tail, 0·15 mm. to 0·23 mm. ; anus to tip of tail, 0·06 mm. to 0·07 mm. ; stylet, 0·008 mm. to 0·009 mm. Proportions, $\alpha = 21$ to 17, $\beta = 11$ to 8, $\gamma = 44$ to 32.

The body tapers slightly in the œsophageal region towards the anterior end and more considerably at the posterior end from the vulva backwards. The tail has a small peg-like tip. The flattened, button-like head is narrower than the end of the body and is marked off from the latter by a very fine constriction. There are no distinct lips, but the surface of the head carries six longitudinal ridges which divide it into six equal areas. In lateral view no oral papillæ are discernible, even under high magnification. The cuticle is finely striated transversely.

The stylet has the typical Tylenchus structure, and is made up of an anterior steeply conical half, attached behind to the posterior cylindrical half, the base of which has three small thickenings. There appear to be muscle bands which are attached towards the base of the stylet and are inserted at the junction of the head with the body. The œsophagus is continued backwards from the base of the stylet. The first part is elongated and has a width of about one-third that of the corresponding region



Agrostis stolonifera.

FIG. 1.—Normal flower, unopened, one anther extruded.

FIG. 2.—Normal flower, opened out to show the various parts.

FIG. 3.—Galled flower, unopened.

FIG. 4.—Galled flower, opened to show the parts. A, anthers; Gl, gall.; I G, inner glume; I P, inner palea; L, lodicules; O G, outer glume; O P, outer palea; Ov, ovary.

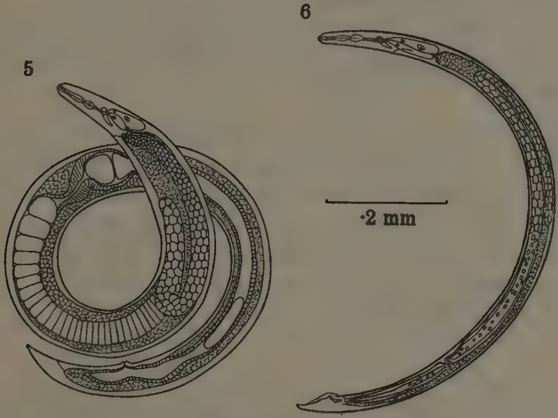
of the body. It contracts a little before expanding into the swollen muscular bulb following which it decreases in width to form a neck region rather narrower than the first part of the œsophagus. This region is crossed by the nerve ring and is frequently folded on itself in mature worms owing to the forward thrust of the ovary in the body. It expands to form the final glandular region of the œsophagus, the substance of which is made up of the three œsophageal glands. The nuclei of all three of these can be distinguished in young adults, but as a rule, only the large nucleus can be seen in gravid worms. The two smaller nuclei lie towards the fore part of this region, whilst the large one is usually nearer the central area. The karysome of each of the smaller nuclei is about half the diameter of the larger one. The lumen of the stylet leads into that of the œsophagus in which it is visible as a clear narrow passage. Very near the base of the stylet the dorsal œsophageal gland opens into it by a short oblique duct. In the muscular bulb there are the usual three crescentric thickenings of the lumen at the base of which the two sub-ventral œsophageal glands open on the ventral side. In the glandular part of the œsophagus, the substance of which completely surrounds the lumen, the latter presents a clear, narrow band on either side which swells out a little posteriorly just before joining the intestine. The latter, as a rule, has a granular appearance and is yellowish brown in colour. There is a short rectum leading to the anus. The excretory pore is found in the vicinity of the broad terminal part of the œsophagus and opens on a slight prominence of the ventral surface of the body.

The vulva is situated far posteriorly on the body and has prominent rounded lips. It opens immediately into the uterine chamber which is continued forward as the uterus proper and posteriorly into a short post-vulval sac which frequently ends in a small mucron as in the female of *T. tritici*. This blind sac usually extends about halfway between vulva and anus. Immediately anterior to the vulva, the rather stout cellular wall of the uterus is expanded for a short distance and is then continued forwards as a tube for a considerable distance. Following a slight constriction it is again enlarged into the receptaculum seminis. Anterior to this the wall again contracts as a short oviduct which connects with the ovary. The latter is continued forward in the body as a long column of cells gradually diminishing in width, and is usually

folded back on itself for some distance, till finally the tip of the organ comes to lie close to the œsophagus.

The eggs are cylindrical in shape with rounded ends and measure about 0·15 mm. long by 0·05 mm. wide.

Male. Principal measurements: length, 1·1 mm. to 1·68 mm.; greatest width, 0·04 mm. to 0·06 mm.; œsophagus, 0·15 mm. to 0·23 mm., anus to tip of tail, 0·06 mm. to 0·07 mm., spicules 0·042 mm., gubernaculum, 0·014 mm. Proportions, $\alpha = 28-23$; $\beta = 9-6$; $\gamma = 23-20$.



Tylenchus agrostis.

FIGS. 5 & 6.—Adult female and male, under low magnification, to show general structure.

The male is smaller and slenderer than the female. The account of the various parts of the alimentary canal given above for the female applies equally well to the male.

The gonad is single and extends forward in the body almost to the beginning of the intestine where it is reflexed on itself for a considerable distance. Approximately the posterior third forms a well-marked *vas deferens* whose junction with the testis is frequently bent into a loop or fold.

Each spicule, when viewed in lateral aspect, is shaped, as shown in fig. 8, like a broad-bladed dagger with a wide handle and a slightly expanded head. There are two ridges running from the tip to the level of the widest part of the blade from which point they become fainter as they run into the handle and join together. The dorsal side of the head is bent over slightly ventrally to form a flattened pointed process. The gubernaculum is simple. The lateral alæ arise a little in advance of the head of the spicules and are inserted close to the tip of the tail as in *T. tritici* and *T. graminis*.

When compared with the spicule of *T. tritici* (fig. 9), that of *T. agrostis* is seen to be somewhat similar in shape, but is shorter and narrower at the widest region. The handle also is longer than in *T. tritici* and the head end has the dorsal side bent ventrally much less than that of *T. tritici*.

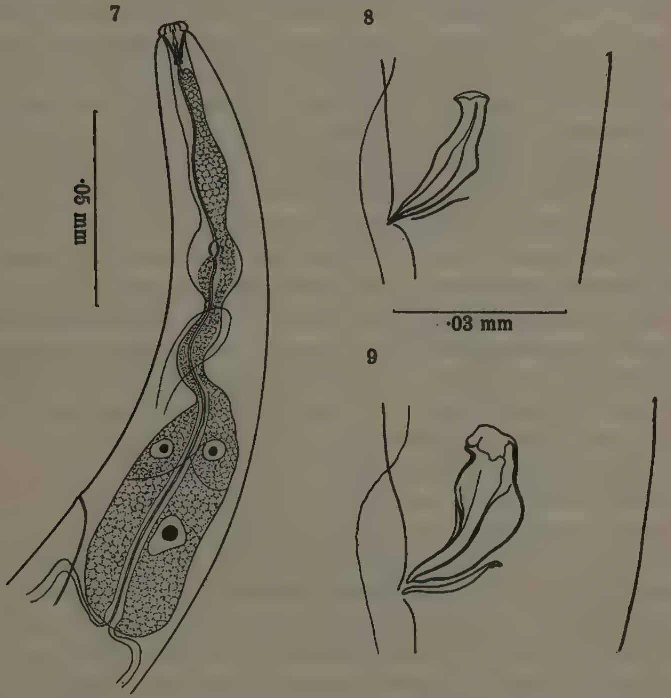
Larvæ. The first stage larvæ measure about 0·55 mm. in length by 0·015 mm. wide. The second stage larvæ are considerably longer and measure 0·75 mm. to 0·8 mm. in length by 0·16 mm. to 0·18 mm. in greatest width. The stylet and typical Tylenchus œsophagus are clearly defined in both first and second stage larvæ which are indistinguishable in appearance from the corresponding larvæ of both *T. tritici* and *T. graminis* (*vide* Goodey, 1927).

Discussion.

As mentioned in the introduction to this paper, all attempts to produce galls in grasses by infecting with those of *T. tritici* from wheat have proved negative. Marcinowski (1909), p. 104, carried out experiments extending over two years, using the following fifteen species of grasses belonging to seven different genera. Although very large numbers of galls from wheat were used, she failed to set up gall formation in any case. *Agrostis capillaris*, *A. stolonifera*, *A. canium*, *Bromus erectus*, *B. pratensis*, *B. secalium*, *Alopecurus geniculatus*, *Festuca ovina*, *F. pratensis*, *F. vulgaris*, *Holcus lanatus*, *Poa annua*, *P. pratensis*, *Phleum Böhmeri*, *P. pratense*.

Leukel (1924), p. 935, states that he carried out similar experiments, using several species of *Agrostis*, *Arrhenatherum*, *Festuca*, *Lolium*, *Phleum* and *Poa*, but failed in any case to set up gall formation.

In the opposite direction, also, the results of experiments are negative. Leukel quotes Bessey as having attempted to infect wheat with galls from the following grasses : *Agropyrum*, *Agrostis*, *Calamagrostis*, *Chætochloa*, *Elymus*, *Sporobolus*, *Trisetum*, but without success. Leukel himself entirely failed to produce galls in wheat from galls in *Calamagrostis*.



Tylenchus agrostis.

FIG. 7.—Oesophageal region of a young adult female to show typical Tylenchus character of stylet and œsophagus.

FIGS. 8 & 9.—Spicule and gubernaculum of *T. agrostis* and *T. tritici* respectively, highly magnified in lateral view.

There is thus good evidence of an experimental character which points to the species being distinct. The very different response on the part of the flowers in *Agrostis* and wheat, to the presence of their respective

parasites, may also be considered as supporting this specific difference between the invading organism. Whereas in *agrostis* there is great lengthening of the glumes and paleæ, in wheat there is none, a point also commented on by Steinbuch.

Steinbuch considered *V. agrostis* to be distinct from *V. tritici* for the following reasons :—(1) Because of its two similar ends, in *V. tritici* one is plumper than the other ; (2) because of the length of the ovary outstretched towards both ends and becoming lost in a gelatinous mass ; (3) because of its lack of a brown colour, possessed by *V. tritici ;* (4) its smaller size ; (5) because of the transparent globules within the body (6) because of the completely different growth and much more delicate structure of the flower parts wherein the worms lie. Of these points, 1, 2, 3 and 5 are negligible from a strict morphological standpoint, and 4 is of somewhat doubtful value in attempting to establish a specific difference between the species in that a mere difference in size might be interpreted as the response of the organism to a different host.

The size difference between the adults of *T. agrostis* and *T. tritici* is, however, very considerable. The females of the latter are much larger than those of *T. agrostis*, and measure from 3·5 mm. to 5 mm. in length, as compared with 1·5 mm. to 2·7 mm. for females of *T. agrostis*. The figures for the respective males are as follows, *T. tritici* 2 mm. to 2·5 mm., *T. agrostis* 1·1 mm. to 1·68 mm. Thus the adults of *T. agrostis* are constantly smaller than those of *T. tritici*.

The writer has shown in an earlier paper (Goodey, 1927) that there are good morphological grounds for considering *T. graminis* (Hardy, 1850) as distinct from *T. tritici*, not only because of its different habitat and smaller size, but because of certain constant differences in the shape of the spicules in the males of each. Similarly it has been shown on p. 206 that the spicules of *T. agrostis* have a different shape from those of *T. tritici*. In addition, therefore, to the biological and experimental considerations already adduced, there is good morphological evidence in support of the distinctness of the two species. Consequently we may consider *T. agrostis* as a valid species.

A word or two may be given on the question of the status of the other species made by Steinbuch, *i.e.*, *V. phalaridis*. He obtained these worms from dried flower galls on the grass *Phalaris phleoides vivipara*, now called *Phleum Böhmeri*. The glumes of the parasitized flower were apparently

of normal size, but within he found one palea enclosing the other as a long pointed cone, and within this was another flattened palea about one quarter the length of the other. Inside this was a brownish, shrivelled, pyriform gall, and when this was soaked in water, worms were obtained, both larvæ and one or two larger forms. The latter were of a brownish colour, but were not so large as the females of *V. agrostis*. Steinbuch thought the head end (actually the tail end, for he mistook the vulva for the mouth) was more pointed than that of *V. agrostis*.

On one of the worms, presumably a male, to judge from his drawing, he noticed a series of transverse stripes or shadows throughout most of the body and he points to this feature as a difference between his two species *V. agrostis* and *V. phalaridis*. Marcinowski (l.c.) p. 128, considers that these markings may be merely ridges on an old shrunken cuticle.

It is clear that the differences noted by Steinbuch and based on the points just mentioned are of little value to-day for the separation of the two species. Further investigation of adult worms from fresh galls on *Phleum Böhmeri* will be necessary before the question can be settled satisfactorily. Houard (1908-13), p. 64, cites Horn-Waren as having found galls in the flowers of this grass. On consulting this paper it was found to have been written by Horn, not Horn-Waren, as given by both Houard and Marcinowski; Waren being the name of his home town. His drawings of affected flowers show elongated glumes and paleæ very similar in appearance to those described above for *Agrostis stolonifera*. The measurements given by Horn for the adult worms from the galls are :—*Females*, 2 mm. to 3 mm. long by 0·2 mm. wide *Males*, 1·2 mm. to 1·6 mm. long by 0·048 mm. wide, stylet 0·009 mm. long, which agree fairly well with those given earlier in this paper for *T. agrostis*. He suggested *T. phlei* as a suitable name in place of *T. phalaridis* owing to the fact that the host had been taken from the genus *Phalaris* and placed in the genus *Phleum*. Galls have also been recorded from the flowers of another species of *Phleum*, namely *Phleum pratense* (Timothy grass). Houard (l.c.), p. 63, gives Hieronymous (1890) as authority though the writer has been unable to consult this paper and verify the reference. From Houard's brief description, however, in this host also the glumes are elongated and thickened.

From these records it is clear that the nematode parasitising the flowers of two species of *Phleum* sets up the same type of reaction in the

host as that in *Agrostis* species. In one of these hosts, the adult worms also are of practically of the same dimensions as those of *T. agrostis*. Consequently, it may not unreasonably be suggested that the parasites are probably identical. If this should turn out to be the case then *T. phalaridis* will become a synonym of *T. agrostis*.

References.

Bastian, H. C., 1865.—"Monograph on the Anguillulidæ, etc.", *Trans. Linn. Soc.*, Vol. xxv.

Bauer, F., 1823.—"Microscopical Observations on the Suspension of Muscular Motions of *Vibrio tritici*," *Philos. Trans. Roy. Soc.*, London.

Bessey, E. A., 1905.—"A Nematode Disease of Grasses," *Science* N.S. Vol., xxi, No. 532, pp. 391-392.

Diesing, C. M., 1850-51.—Systema Helminthum, Vol. 2, p. 132.

Dujardin, F., 1845.—Histoire naturelle des helminthes

Ehrenberg, C. D., 1838.—Die Infusionthierchen als Vollkommene Organismen.

Goodey, T., 1927.—"On *Tylenchus graminis* (Hardy, 1850), Marcinowski, 1909," *J. Helm.*, Vol. v., No. 3, pp. 163-170.

Hieronymous, G., 1890.—"Beiträge zur Kenntniss der europäischen Zoocecidien und der Verbreitung derselben," *Jahresb. schles Ges. f. vaterl. Kultur*, Bd. 68.

Horn, P., 1888.—"Die Aelchen-Gallen auf *Phleum Boehmeri* Wibel," *Arch. Fr. Naturg. Mecklenb.* 42, pp. 139-156, published 1889. Güstrow.

Houard, C., 1908-13.—Les zoocécidies des plantes d'Europe et du Bassin de la Mediterranée. Paris.

Leukel, R. W., 1924.—"Investigations on the Nematode Disease of Cereals caused by *Tylenchus tritici*," *J. Agric. Res.*, Vol. xxvii, No. 12, pp. 925-955.

Marcinowski, K., 1909.—"Parasitisch und semiparasitisch an Pflanzen lebende Nematoden," *Arb. biol. Anst. Land-u. Forstw.*, Bd. 7, H. 1.

Steinbuch, J. G., 1799.—"Das Grasælchen, *Vibrio agrostis*," *Der Naturforscher* xxviii, pp. 233-259.

Stiles, C. W. and Hassall, A., 1920.—Index-Catalogue of Medical and Veterinary Zoology, Roundworms.

Plate.

Photograph of three panicles of *Agrostis stolonifera*. On the left, an open panicle, all flowers galled, with long glumes. In the centre, an open panicle, many normal flowers with small glumes and a few galled flowers with long glumes. On the right, a compact panicle, all flowers galled with long glumes.

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp.* 197-210. *Facing p.* 210.

Agrostis stolonifera infested with *Tylenchus agrostis.*

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp*. 211-222.

On Variations in size of the Nematode Worm *Rhabditis succaris* N. SP., produced by Different Culture Media.

By PHYLLIS A. CLAPHAM, B.Sc.

(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine*).

DURING the past year the writer has been making routine examinations of the Nematode fauna of filter beds, using for this purpose clinker collected from the filter beds of Brent Sewage Farm.

The zoogloeal film on the clinker is rich in animal life and the Nematode worms are abundant in both numbers and species. It is interesting to note in this respect, however, the great variations which occur. The genera themselves are fairly constant and some species also occur regularly. On the other hand the species which occur in fewer numbers show great variability. *Rhabditis succaris*, some notes on which appear below has been observed once and then in fair numbers. Several species have cropped up once only and then only a single specimen or a few individuals have been observed.

In the course of these routine examinations many species of Nematodes have been observed and one in particular, *Rhabditis succaris*, has proved itself interesting in the production of different strains in the laboratory. It has been impossible however, definitely to classify it among those species already made known and hence it has been decided to consider it, temporarily at least, as a new species. A description of its structure and a note on its biology follows.

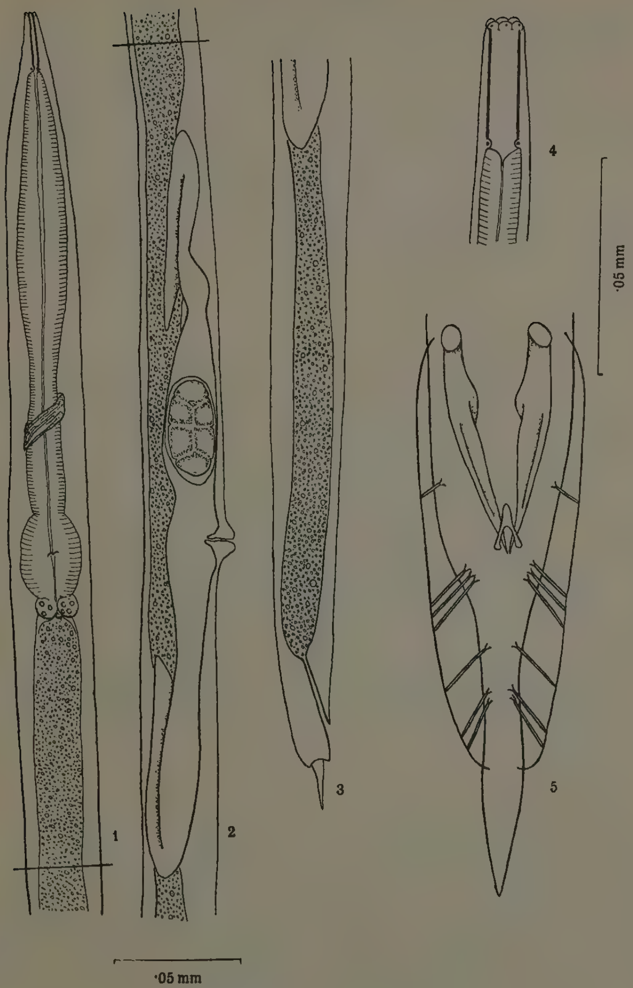
The writer is indebted to Professor R. T. Leiper, under whose supervision the work has been carried out, for much assistance in many ways.

The Structure of the Adult.

The mature *Rhabditis succaris* is a slender worm which always shows active movements. For superficial examination they were mounted in a hanging drop prepaiation and put into a Frigidaire Cooling Apparatus for 5 to 10 minutes where a temperature of abour 7° C. was registered. This cold atmosphere stupified the worms for a period of time long enough for rough identification but they revived in the laboratory within 3 to 5 minutes. In order to examine them closely it was found necessary to kill them. The method adopted here was to heat the worms gently and when they had straightened out to flood them with hot Ditlevsen's Glycerine—Alcohol fixative. This method had the advantage that the worms died in an extended condition and the hot fixative permeated the tissues immediately. Death and fixation supervened so rapidly that in a large percentage of cases no abnormal post-mortem changes, such as great swelling up of the cuticle, were visible.

Rhabditis succaris possesses the characters of the genus *Rhabditis*—that is to say there is clearly visible the elongated buccal cavity and double bulbed œsophagus. In the male the tail is surrounded by a pair of caudal alæ supported by rays.

The cuticle is perfectly smooth and even, being devoid of striations and bristles. It is thin and covers the whole body regularly. The mouth pore is terminal and is surrounded by six small but well-defined lips, each bearing a small papilla. No bristles have been so far observed to be connected with these papillæ. The mouth leads into a straight buccal cavity about 25μ in length ; anteriorly in the chitinised wall there may generally be seen by careful focussing, 3 or 4 slight thickenings which seem to be of the nature of circular ridges. These are however somewhat ill-defined. Posteriorly and lying at the base of the buccal cavity there is a distinct thickening. In optical section therefore the buccal cavity takes on the typical Rhabditoid appearance of a pair of straight lines each followed by a small dot. No teeth or definite cuticular thickenings were to be observed in the buccal cavity. Following this is the œsophagus about 175μ to 225μ in length. This is typical for the genus with two bulbs, the posterior one of which is strongly muscular and contains the chitinised valvular apparatus. The anterior bulb is well-defined. The nerve ring is placed close behind the anterior bulb



Rhabditis succaris sp. nov.

FIGS. 1-3.—Entire female worm, in three divisions. (Lateral view.)
FIG. 4.—Anterior end showing buccal cavity.
FIG. 5.—Tail and bursa of male worm (ventral view).

and under favourable conditions it was possible to see in some specimens a small excretory pore at the level of the nerve ring.

Immediately following the œsophagus are three large cells. These are generally full of large dark granules and are easily distinguishable from the intestinal cells which follow. It may be that they have a digestive function, though as yet no definite changes have been seen in these cells. The intestine is straight, composed of flat cells usually full of large oily granules and opens to the exterior by a short rectum.

The tail is short and tapers quickly to a point ; it is slightly longer in the male than in the female.

Male Reproductive System.

The male is smaller than the female and very abundant. In a count of some 200 mature worms taken at random 45 per cent. were found to be males. The testicular tube is single and reflexed anteriorly. The vas deferens becomes swollen in a mature specimen and is filled with a mass of sperm thus taking on the function of a receptaculum seminis. There is a pair of stout, similar and equal spicules about 37μ in length from tip to tip and a single accessory piece. The head of the spicule is well developed and a strong extensor muscle is attached here. The shaft is broad and grooved forming an efficient channel for the sperm. The bursa is well formed ; the alæ being supported by 7 pairs of rays. Of these 1 is pre-anal and the others post-anal : three arise in a group close behind the anus and the posterior pair arise close together and are directly sharply backwards. The tail passes beyond the alæ for a short distance.

Female Reproductive System.

The female genital tubes are typical for the genus—that is to say they are paired, opposed and reflexed. Distally there is a mass of cells constituting the ovary, the duct then becomes hollow and finally acquires a well defined wall—this region being an oviduct. The worm is oviparous but usually there occurs a certain amount of segmentation within the oviduct before the egg is deposited—8 or 16 cells being an average stage of development found in the oviduct. Maturation and deposition takes place rapidly once the worm has become mature—20 or more eggs having been laid by a mature female in the night. Occasionally a segmenting egg may be visible in both the uteri but generally only one is present.

When two are present one is always in a further advanced state of development than the other. The egg is an ovoid structure, slightly flattened on one side and measuring from $62 \cdot 5\mu$ to 50μ in length by 25μ to 10μ in breadth.

Male and female are frequently to be seen in copulation and that this occurs early in life is evident from the fact that it is almost impossible to find a female worm that is not already impregnated with a large mass of sperm. The sperms on entry pass along the oviduct and come to lie in a wide region close behind the ovary. The ova must therefore pass through this store, where they become fertilised, on their way down the oviduct.

Culture Methods and Formation of Different Strains.

Many media were used in an attempt to cultivate a pure strain of this species. Its occurrence in the zoogloeal film round the clinker on the filter beds is very spasmodic. When the material is collected one or more species of *Rhabditis* may be visible, but this particular species was not noticed until the material had been standing in the laboratory for 6 weeks or more. By this time the fauna had changed considerably. The bulk of the Protozoa had disappeared as also had many of the Nematodes and there were no segmented worms or insect larvæ left. Rotifers were still common and there were many bacteria. The fluid had a lower oxygen requirement having been much oxidised in the intervening weeks and hence the green algæ, which are normally present in small numbers, had taken the opportunity of growing abundantly. The medium had therefore lost most of the characters of sewage material and had taken on more of the characters of a fresh water pond.

A pure culture (A) was made in June, 1929, from a single mature female in a petri dish with distilled water. Some débris and decaying matter was added at the same time and formed a sediment at the bottom of the dish. As the culture did not thrive rapidly some pieces of clinker were added and the whole culture kept in a cool, dark place, for it was noticed that the worms were always more abundant in the shady portions of the dish. They then multiplied rapidly and by the end of July, 1929, the culture was healthy and active. Several sub-cultures were formed with a view to discovering how the worms were affected by change of food and conditions.

Attempts were made to culture the worms in soil, fæces and charcoal, on plain and nutrient agar plates, in glycerine and albumen and in solutions rich in bacteria. The first positive result was obtained on the nutrient agar plate and later it was found that the same good result was to be obtained by means of a dilute solution of " Lemco "—the brand of meat extract which had been used to make the nutrient agar plates. A certain green alga—a species of *Scenedesmus*—was introduced with the worms and throve abundantly as the dish was left for some time on the laboratory bench in the light. This culture will be referred to as Culture (B).

In an attempt to classify the worm some weeks later recourse was made to the formula first introduced by de Man. His symbols are :—
L. representing the total length of the worm in mms.

α. ,, ,, proportion $\dfrac{\text{Length of body}}{\text{Greatest breadth}}$ *i.e.*, relative breadth.

β. ,, ,, ,, $\dfrac{\text{Length of body}}{\text{Length of oes}}$ *i.e.*, relative length of oes.

γ. ,, ,, ,, $\dfrac{\text{Length of body}}{\text{Length of tail}}$ *i.e.*, relative tail length.

V. denoting the distance of the vulva from the anterior end, expressed as a percentage of the total length of the body. This formula is useful for comparing worms of the same species for it is generally found that the values remain constant, within small limits, for members of the same species. In this case, however, a very decided discrepancy was noticed. The measurements of the worms taken from the original culture (A) being much shorter than those taken from the " Lemco " culture (B), and as this difference seemed to remain constant in each culture, 500 worms, 250 of each sex, were accurately measured from each petri dish and the results compared. Only mature, healthy worms were used for measurement and these were killed by the application of very gentle heat. Any worms which showed great swelling up of the cuticle were discarded. No other post-mortem changes were visible. If any shrinkage had occurred, however, then one may expect the error to be the same for all the worms as they had all been treated alike. Measurement proceeded within a few minutes of fixing.

As the number of measurements was bulky, each symbol was considered separately and the actual mean of the results and the standard deviation from the mean were calculated and considered. The females and males of each strain could then be compared. The writer would like to express her thanks to Miss H. M. Woods for much help in the application of statistical methods to these results.

In the accompanying table the results of the measurements have been set out :—

	Actual Mean.		*Standard Deviation.*	
L.—Length of the Body.				
	Culture A ♀ 0·97	Culture A ♂ 0·88	Culture A ♀ 1·83	Culture A ♂ 1·55
	„ B ♀ 1·39	„ B ♂ 1·18	„ B ♀ 3·06	„ B ♂ 3·09
α.—Relative Breadth.				
	Culture A ♀ 28·96	Culture A ♂ 28·75	Culture A ♀ 2·12	Culture A ♂ 2·35
	„ B ♀ 30·15	„ B ♂ 30·01	„ B ♀ 2·29	„ B ♂ 1·81
β.—Relative Length of Oesophagus.				
	Culture A ♀ 4·75	Culture A ♂ 4·74	Culture A ♀ 2·01	Culture A ♂ 2·12
	„ B ♀ 4·96	„ B ♂ 4·95	„ B ♀ 2·29	„ B ♂ 2·04
γ.—Relative Length of Tail.				
	Culture A ♀ 28·49	Culture A ♂ 26·98	Culture A ♀ 2·32	Culture A ♂ 2·55
	„ B ♀ 30·01	„ B ♂ 26·85	„ B ♀ 2·48	„ B ♂ 2·08
V.—Position of Vulva.				
	Culture A ♀ 57·34%		Culture A ♀ 2·26	
	„ B ♀ 57·72%		„ B ♀ 1·69	

From these results it can be seen that the actual length of the worms taken from the original culture (A) is consistently less than those from the "Lemco" culture (B). The same result is obvious whether the male or the female be considered. The relative measurements do not show any great difference. With the exception of the food material offered, the worms were cultivated under very similar conditions ; hence one may draw the conclusion that the dilute solution of "Lemco" has had the effect of producing a giant race. The worms in the first petri dish fed entirely on débris and decaying matter whereas the others had the opportunity of an abundant supply of protein and also of green algæ which had grown there. In the dish the *Rhabditis* could often be seen ingesting separated cells of *Scenedesmus*. They fed voraciously until the intestine was packed with cells. Some digestion occurred rapidly

so that the green colour was lost early but the cells following the œsophagus were not observed to alter appreciably in composition or appearance during this process.

Another interesting point to notice is that the " Lemco " strain shows far greater variability than the other strain. This perhaps is not surprising, as this strain has been definitely cultivated on abnormal food which the worms would not meet with under natural conditions.

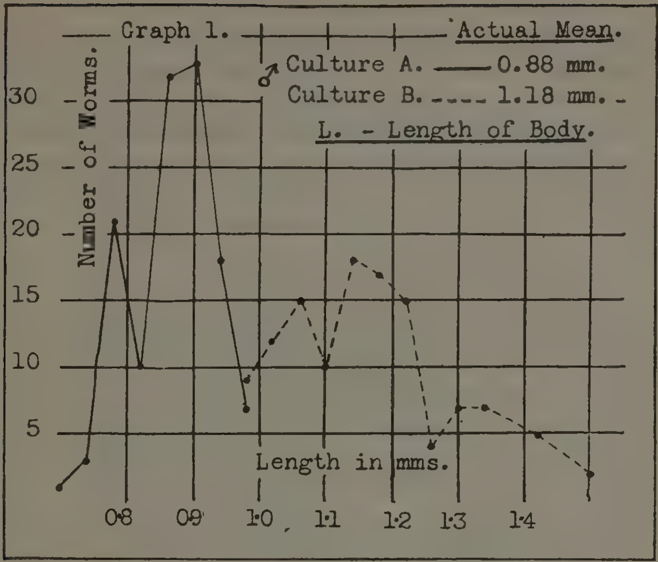
A control culture (C) was set up using worms of the " Lemco " strain (B)—that is the worms were all large. These were placed in a petri dish with organic débris and pieces of charcoal and the whole kept in the dark. This dish was therefore kept under the same conditions as was the original strain (A).

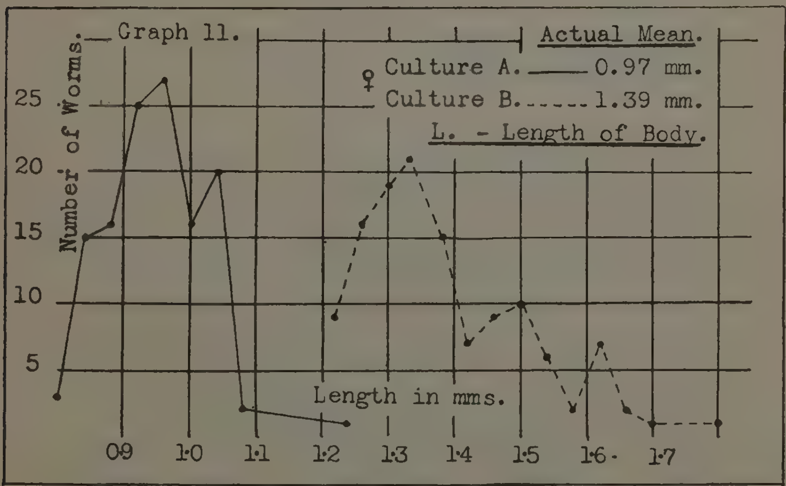
After the culture had been proceeding for about three months 500 worms, equal numbers of both sexes were removed and measured as before. An interesting result was apparent : the dimensions of these worms approximated closely to those of culture (A). Only the length of the worms was considered as the relative measurements had showed little variation.

In the female L varied from 0·74 mm. to 1·22 mm. and in the male from 0·70 mm. to 0·94 mm. : the actual means being 0·90 mm. (female) and 0·83 mm. (male) and the standard deviations from the mean being 2·1 mm. and 0·53 mm. respectively.

This result bears out the statements made by Henneberg in 1900 in writing of vinegar eelworms when he says that " the size of the eelworms varies according to the food " and by Conte also in 1900 when working with *Rhabditis monohystera* when he also noted the definite influence which the culture medium had on the size of the worms. He says " Cultivé sur colle de pâte pendant un temps assez long, ses dimensions se modifient avec la valeur nutritive du milieu, sa taille varie du simple au double (de 1·2 mm. par exemple à 2·35 mm.)." He also noted a change from oviparity to viviparity when the worms were transferred from a poor to a rich medium but the writer has constantly observed 1 or 2 ova in her cultures. The reproductive activity of the worm has so far remained constant.

The fluctuations in the sewage fauna are very great and the writer has unfortunately only come across this particular species once since the work described above has been under way. On this particular occasion





GRAPHS 1 and 2.—Length of male and female worms, illustrating the distribution round the mean.

there was found an unhealthy female which died without producing another generation. Hence it is impossible to compare the laboratory bred worms with worms taken directly from the natural habitat. It is not unreasonable to suppose, however, that organic débris approximates more closely to the natural food than does a solution of protein containing green algæ. This, coupled with the fact that the original strain is much less variable, suggests that the measurements of worms taken directly from the filter beds, would closely approximate to those fed on organic débris.

With such results as have been catalogued already it will be seen that it is difficult to apply the usual formula of de Man to this worm. While the relative measurements are not appreciably different, the length of the worms from the two cultures remains distinct. Here there is practically no overlapping of results; one worm only among the females in culture (A), which was 1·24 mm. in length, reaches the length of the worms in (B). A very similar result is obtained among the males. The largest male from (A) is the same length as the smallest in (B), and in both cases the mean of the results is widely different. The accompanying graphs accentuate this difference.

The following table indicates as nearly as possible the measurements of the species *Rhabditis succaris* :—

Female.

L.—	0·97 mm.	(0·80 to 1·24)	or	1·39 mm.	(1·22 to 1·78)
α.—	28·96	(27·50 to 31·50)	or	30·15	(27·50 to 32·00)
β.—	4·75	(4·30 to 5·10)	or	4·96	(4·20 to 5·30)
γ.—	28·49	(25·50 to 30·00)	or	30·01	(27·00 to 32·00)
V.—	57·34%	(53·00 to 59·00)	or	57·72%	(56·00 to 59·00)

Male.

L.—	0·88 mm.	(0·70 to 0·98)	or	1·18 mm.	(0·98 to 1·50)
α.—	28·75	(27·00 to 31·50)	or	30·01	(28·59 to 31·50)
β.—	4·74	(4·20 to 5·10)	or	4·95	(4·30 to 5·20)
γ.—	26·98	(22·50 to 28·50)	or	26·85	(23·50 to 28·00)

Summary.

(1) During the routine examination of the fauna of samples of clinker removed from the filter beds of Brent Sewage Farm several species of *Rhabditis* were met with, one at least of which cannot be relegated to any species as yet described. A new species is therefore suggested for its reception and the name *succaris* has been given to suggest its oiigin from the sewage farm.

(2) An account is given of its structure. While being typical for the genus, the lips and bursal rays do not agree with any known species.

(3) Two distinct strains were reared by means of different culture media, differing quite definitely in length. A thousand worms were measured and these differences were found to remain very constant. The relative measurements have not been materially altered.

(4) The length of the large worms has been diminished by means of a return to the original food.

(5) The cultivated strain shows greater variability than the original strain.

(6) Two graphs and a table summarising the results are shown.

References.

Cobb, N. A., 1918.—" Filter Bed Nemas. Contributions to the Science of Nematology," VII.

Conte, A., 1900 A.—" De l'influence du milieu nutritif sur le développement des Nématodes libres," *C. R. Soc. Biol.*, Paris, LII. (*W.L.* 6630.)

— 1900 B.—" Sur les conditions de ponte des Nématodes," *C. R. Soc. Biol.*, Paris, LII. (*W.L.* 6630.)

Goodey, T., 1930.—" On the Presence of Fats in the intestinal wall of Nematodes," *J. Helm.*, vol. VIII., p. 2.

Man, J. D. de, 1884.—" Die frei in der reinen Erde und in süssen Wasser lebenden Nematoden der Niederlandischen Fauna."

Maupas, E., 1899.—" La mue et l'enkystement chez les Nématodes," *Arch. zool, exp. gén.*, (3) 7. (*W.L.* 1915.)

— 1900 —" Modes et formes de reproduction des Nématodes," *Arch. zool. exp. gén.*, (3) 8. (*W.L.* 1915.)

MICOLETZKY, H., 1921.—" Die freilebenden Erd-Nematoden," *Arch. Naturgesch.*, Jahrg. 87, Abt. A. Heft, 8-9. (*W. L.* 1782.)

PETERS, B. G., 1930.—" A Biological Investigation of Sewage," *J. Helm.*, vol. VIII., p. 3.

— 1930.—" Some Nematodes met with in a Biological Investigation of Sewage," *J. Helm.*, vol. VIII., p. 3.

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp.* 223-228.

On the Differential Diagnosis of the Larvæ of some Helminth Parasites of Sheep and Goats

By D. O. MORGAN, M.Sc., Ph.D.

(*Senior Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine*).

INTRODUCTION.

In the majority of cases it is almost impossible to identify the species of helminths in our domestic stock by an examination of the eggs found in their droppings. In the case of sheep and goats, this is particularly so since these hosts harbour several worms with eggs which are, apparently, identical in shape, size and stage of development.

It is true that the eggs of such types as flukes, tapeworms and whipworms can be picked out fairly easily, but the forms belonging to the Strongyloidea in particular produce eggs which are not so readily identified and it is these species which occur most commonly in natural infections.

It is the common practice where a flock of sheep is doing very badly and where worms are suspected to be the cause, to examine a dead animal if one is available, or to kill one of the poorest, in order to ascertain the type of infection present. The method must remain in many cases as the most efficient and quicker means of diagnosis. Where, however, it is desirable to have some idea of the species harboured by a flock and particularly for experimental purposes, some method whereby this can be ascertained by a fæcal examination is most valuable.

Although an examination of the eggs is of little value for identifying many species, one may go a step further if one examines the infective larvæ obtained from cultures of these eggs. Veglia (1924 and 1928) has shewn that the larvæ of some of the commoner forms found in sheep in South Africa can be determined by the length of the tail. He found that magnifications of 50 to 70 were sufficient for this purpose, and that even 35 diameters would be adequate to the accustomed eye. There is no doubt that with constant practice one would be able to identify a number

of larvæ under these low magnifications ; it is not always easy, however, to appreciate small differences in length of the tail when the larvæ are active, and particularly when the culture does not contain an abundance of one or two species, which can be easily identified and used as a standard for comparison.

So far, the writer has only been able to study the larvæ of some of the commoner worms of sheep and goats in this country. Several of the members of the family Trichostrongylidæ have not been examined owing to the great difficulty in obtaining pure cultures of each species.

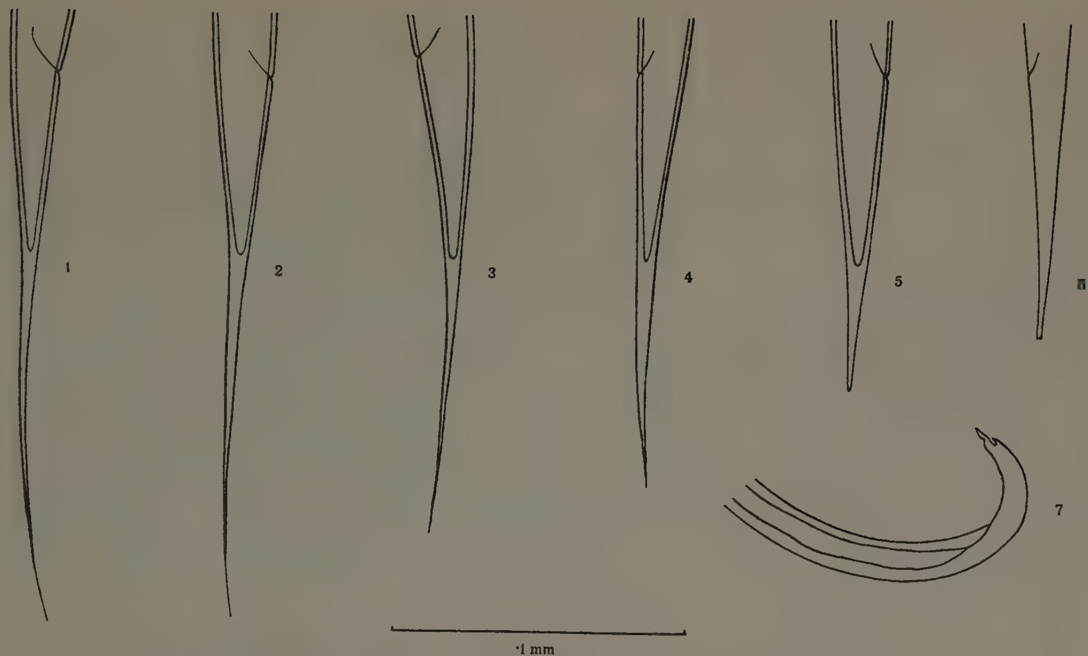
Technique.

Eggs obtained by teasing out female worms of each species were cultured at 25° C. in sterilized sheep's fæces mixed with animal charcoal or sterilized soil. In some species the infective larvæ climbed on to the cover of the petri-dish and were collected by flooding this with warm water ; the non-climbers were obtained by flooding the culture. Some of the larvæ were then placed on a slide and killed by gentle heat, care being taken not to apply too much heat, since the larvæ tend to contract within the sheath. The slide was then examined under the microscope and the length of the tail of each larva was then determined, excepting larvæ which had other distinguishing characters.

Since the position of the anus is often difficult to determine it was found more convenient to measure the distance from the tip of the tail of the third stage larvæ to the tip of the tail of the second stage. This gave quite a reliable measurement, provided no undue contraction had taken place in killing the larva. By using a No. 2 micrometer eye-piece and a $\frac{1}{6}$-in. objective this measurement can be quickly obtained. In fact the actual length of this region in millimeters need not be calculated once the number of divisions on the micrometer has been determined for each species. In this paper, however, the actual measurement is given and, as previously stated, this has not been taken from the anus, but from the tip of the tail of the third stage to that of the second stage.

Larval Characters.

Œsophagostomum venulosum and *Chabertia ovina* have larvæ which are very characteristic, but the writer has been unable to differentiate between these two species. In both, the larvæ are fairly broad and have a long



FIGS. 1-6.—Tails of infective larvæ of parasitic Nematodes in the following order:—
Œ. venulosum, *C. ovina*, *B. trigonocephalum*, *H. contortus*, *O. circumcincta*, *S. papillosus*.

FIG. 7.—Tail of free living larva of *M. capillaris*.

whip-like tail measuring 0·117 mm. to 0·13 mm. Another characteristic feature is the peculiar structure in the anterior end of the œsophagus. This has already been described by Goodey (1924) in the infective larva of *Œ. dentatum*, and also by Cameron (1926) in that of *C. ovina*. The writer has found a similar structure in the larva of *Œ. venulosum*. Although the details of this region are difficult to determine it is fairly clear that the lumen of the œsophagus at this point swells out until it becomes almost as wide as the œsophagus and that the cuticular lining is drawn into thin strands. The occurrence of this structure in the larvæ of species belonging, at least, to two of the genera of Œsophagostominæ is of taxonomical interest. From the point of view of larval identification, this structure enables one to separate the larvæ of large intestine parasites from those found in other parts of the alimentary canal.

The next, as regards length of tail, is the larva of *Bunostomum trigonocephalum*. In this case the tail is also slender and measures 0·09 mm. to 0·1 mm.

Hæmonchus contortus has also a larva with a long tail, but is slightly shorter and not so slender as that of *B. trigonocephalum*. It measures 0·07 mm. to 0·084 mm.

Of the species which produce larvæ with a short tail the writer has, so far, only studied *Ostertagia circumcincta*. In this larva, the tail has a length of 0·04 mm. to 0·045 mm. and is somewhat conical in shape.

It is probable that the other small members of the Trichostrongylidæ have also short tails, since cultures of fæces from sheep containing these worms have yielded many larvæ of this type. Veglia (1924) has shewn that this is the case in *Trichostrongylus instabilis* and *T. extenuatus*. The tail of the larva of the latter species, according to Veglia's drawing, is much shorter than that of *O. circumcincta*.

Strongyloides papillosus and *Nematodirus filicollis* produce larvæ which can be identified by characters other than length of tail. In the former species the œsophagus of the infective larva is about half the length of the body, and it also has a bifid tail. In the latter species the larva has an exceptionally long tail, and moreover the tail of the third stage larva in this case is characteristic in having a dorsal and a ventral lobe between which arises a rod-like process. Both these species, however, can be easily identified in the egg-stage.

The two most common lungworms of sheep in this country are *Muellerius capillaris* and *Dictyocaulus filaria*, and the larvæ from each of these are easily identified. By a Bærmann extraction of fresh fæces the larvæ are readily obtained since the eggs of both species are generally hatched out before leaving the body of the host. The larva of *M. capillaris* is very small and has an undulating tail with a short dorsal process. In the case of *D. filaria* the infective larva usually remains in the sheath of the first and second stage and may be identified by this character. Further, the first stage larva has a knob-like structure terminating the anterior end and is a well-marked character.

Egg Characters.

Of the species which can be identified by an examination of the eggs the following may be referred to :—

Fasciola hepatica has an oval shaped egg provided with an operculum.

Moniezia spp. have globular or almost triangular shaped eggs containing a characteristic pyriform apparatus.

Nematodirus filicollis has by far the largest egg of the Strongyle type and measures 0·175 mm. to 0·2 mm. by 0·1 mm.

Strongyloides papillosus has a very small egg, measuring 0·04 mm. to 0·06 mm. by 0·02 mm. to 0·025 mm. It has a very thin shell and is usually embryonated when passed in the fæces.

Cooperia curticei has a slightly larger egg than that of *S. papillosus* and is not embryonated when passed in the fæces. It is, however, somewhat smaller than the eggs of other Trichostrongyles found in sheep. It measures 0·063 mm. to 0·07 mm. by 0·03 mm. to 0·032 mm.

Trichuris ovis has a barrel-shaped egg with a plug at each pole. It measures 0·07 mm. to 0·08 mm. (including the opercula) by 0·03 mm. to 0·035 mm.

Capillaria longipes has an egg similar to that of *T. ovis*, but much smaller. It measures 0·045 mm. to 0·05 mm. (including the opercula) by 0·022 mm. to 0·025 mm.

Ascaris ovis has an egg which is characteristic in having a shell with a coarsely tuberculate surface.

Skrjabinema ovis has the typical Oxyuris egg which is convex on one side and almost concave on the other.

Remarks.

It is possible to diagnose the presence of most of the common species of helminths found in sheep and goats by the following methods :—

1. An examination of the eggs found in the fæces.
2. A Bærmann extraction of the fæces and an examination of the larvæ thus obtained.
3. An examination of the infective larvæ obtained from cultures of the fæces.

This study is by no means complete, and it is probable that further work will reveal other morphological differences between the larvæ of these parasites. Such differences would, however, be of little value in routine diagnosis if it requires the higher powers of the microscope to reveal them. The possibility of using biological differences in separating the larvæ must not be disregarded. An example of this is the variation in the migratory habits of several species. *S. papillosus* larvæ climb out of the culture in about three days and *H. contortus* a day or two later. The larvæ of *B. trigonocephalum*, on the other hand, can only, as a rule, be recovered from the culture itself.

References.

Cameron, T. W. M., 1926.—" On the Morphology of the Free Living Larvæ of *Chabertia ovina*," *J. Helm.*, Vol. iv, Nos. 4-5, pp. 185-190.

Goodey, T., 1924.—" The Anatomy of *Œsophagostomum dentatum* (Rud.), a nematode parasite of the Pig, with Observations on the Structure and Biology of the free living Larvæ," *J. Helm.*, Vol. ii, No. 1, pp. 1-14.

Veglia, F., 1924.—" Preliminary Notes on the Life History of *Œsophagostomum columbianum*," *9th and 10th Rep. Vet. Edu. Res., S. Africa*, pp. 811-823.

,, 1928.—" Œsophagostomiasis in Sheep," *13th and 14th Rep. Vet. Edu. Res., S. Africa*, Part ii, pp. 755-797.

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp.* 229-238.

On a Lungworm, *Crenosoma potos* n. sp., from the Kinkajou, *Potos flavus* (Schreber).

By J. J. C. Buckley, M.Sc.

(*Milner Research Student, Department of Helminthology, London School of Hygiene and Tropical Medicine*).

The lungworm genus *Crenosoma*, founded by Molin in 1861, was re-described in 1928 by Skrjabin and Petrow. To the two existing species, *C. striatum* (Zeder, 1800) from the hedgehog (*Erinaceus europæus* L.) and *C. vulpis* (Dujardin, 1845) from the fox (*Vulpes vulpes* L.), these writers added a third species, *C. taiga*, from the lungs of *Kolonocus sibiricus* (Pall.) in Russia. A generic diagnosis based on a study of these three species was supplied, and the wolf (*Canis lupus* L.) was recorded as a new host for *Crenosoma vulpis*.

The following description is concerned with a fourth species from the bronchial tubes of *Potos flavus* (Schreber), which died in the Zoological Gardens, London.

In view of certain structural differences presented by *Crenosoma potos*, the generic diagnosis drawn up by Skrjabin and Petrow is insufficient to include it ; but until further species are found that will enable these differences to be estimated truly, it seems inadvisable either to amplify the diagnosis to accommodate *C. potos*, or to erect a new genus for it.

Description of *C. potos*, n.sp.

The transverse folds in the cuticle are more or less equally spaced and occupy only the anterior part of the body, for a distance that is not definitely limited posteriorly. The folds do not cease abruptly, but posteriorly they become less distinct and may disappear altogether. This is particularly noticeable in the male. The cuticle is ornamented

with very delicate parallel longitudinal ridges, whose length is in accordance with the distance between the folds. They give a crenated appearance to the margin of the fold where the cuticle is reflected back (Fig. 9.) The ridges are present over the whole surface of the worm, except immediately posterior to each fold, which is smooth for a short distance (Fig. 9). Posteriorly, where the folding has ceased, the ridges are interrupted at regular intervals by transverse smooth areas of cuticle which represent the straightened-out folds.

In the head region the cuticle forms a collar which is incomplete laterally (Figs. 6 and 7). At the level of the excretory pore there is a deep groove in the cuticle extending around the worm for one-half its circumference. The body in the œsophageal region is considerably flattened laterally.

The mouth possesses a pair of bilobed lips dorso-ventrally opposed, which are with difficulty observed, except from an end-on aspect (Fig. 6). There are eight minute papillæ, one on each lobe, and two at the base of each lip. An insignificant mouth cavity leads to the œsophagus, whose lumen is lined with chitin. The œsophagus is club-shaped, increasing gradually in diameter posteriorly. There are no valves at its junction with the intestine; the anterior end of the intestine is merely inserted inside the œsophagus for a short distance.

The intestine is a very solid thick-walled structure. Its cells are not apparent, but it contains numerous oval nuclei with sparse chromatin.

Ventral to the œsophagus and intestine is a large pair of cervical glands, of an elongated pear-shape. They may be indented or misshapen due to contact with one another or with the intestine. They are very granular and nuclei are not apparent.

The Male.

The male is 12 to 14 mm. long by 0·4 mm. to 0·52 mm. in maximum diameter. It begins to taper anteriorly near the junction of the first and second fourths of the body, and posteriorly it tapers slightly about 2·5 mm. from the bursa.

The cuticular folding is very definite for the first 3 to 4 mm., after which it becomes gradually more vague, but it is represented almost as far back as the bursa by the transversely interrupted ridges. The first 30 to 40 folds are spaced at about 0·1 mm., and the subsequent folds (or their representations) are more distant from one another.

The excretory pore has an average distance of 0·14 mm. from the anterior end of the worm, and the cervical glands are from 1·5 mm. to 1·6 mm. long.

The œsophagus averages 0·35 mm. in length by 0·06 mm. in maximum breadth.

The bursa is very well developed. It is large and bell-shaped and has four lobes, two lateral, a dorsal and a ventral. The dorsal and ventral lobes are subdivided into two by a median notch. The notches formed by the junction of the lateral lobes with the ventral and the dorsal lobe are approached respectively by the tips of the ventral rays and the postero-lateral rays. The rays have stout bases but are long and tapering. Their disposition, relative length and origin should be evident from the ventral and lateral aspects of the bursa depicted in Figs. 4 and 5.

The spicules are equal and average 0·97 mm. in length. In lateral aspect they are faintly S-shaped and appear straight dorso-ventrally. They are closely applied to one another along their whole length. Their diameter is greatest near the root, whence they taper to the distal end where each spicule terminates in a definite knob. They are tubular in structure, and each spicule is enveloped in a delicate membrane and possesses an insignificant "dorsal process" (Fig. 10). The accessory piece is 0·28 mm. long, composed of coarsely stippled chitin and its shape fairly constantly resembles that depicted in Fig. 4.

The Female.

The female is 23 mm. to 26 mm. long by 0·52 mm. to 0·6 mm. in maximum diameter. Anteriorly the body begins to taper about 4 mm. to 5 mm. from the anterior end and posteriorly it narrows somewhat abruptly about 1 mm. from the tip of the tail.

The cuticular folds are very distinct from the first 4 mm. to 5 mm. of the body where they are about 1·5 mm. apart. The subsequent folds become more or less gradually obliterated. They may be present to some degree as far back as the anus.

The excretory pore is 0·14 mm. from the anterior end of the body and the cervical glands 1·7 mm. long, being slightly longer than in the male.

The œsophagus is 0·4 mm. long by 0·08 mm. in maximum diameter.

The tail is 0·3 mm. to 0·4 mm. long and there is a pair of lateral papillæ 0·1 mm. from the tip, which often appear almost dorso-ventral in position due to the tail being twisted about its longitudinal axis.

The vulva is a much enlarged organ 0·75 mm. long by 0·5 mm. maximum diameter, situated about 1 mm. to 3 mm. anterior to the middle of the body. It consists of an outer envelope of cuticle which is continuous with the body cuticle, and this is invaginated distally to form a narrow central lumen into which the vagina opens. The space enclosed between the inner tube and the outer envelope is filled with a gelatinous material through which are scattered spherical cysts of 0·01 mm. to 0·02 mm. in diameter. These resemble " chalk bodies " in appearance, but when exposed to pressure they rupture and fluid emerges. The shape of the vulva is characteristically as depicted in Fig. 2. It is usually directed posteriorly at an angle of about 45° to the body.

The wall of the vagina is slightly chitinised, but its distal end is surrounded by a stout collar of chitin.

The ovejector lies parallel to the longitudinal axis of the worm. The pars ejectrix is kidney-shaped and is very much larger than either pars haustrix, which are short and narrow.

At their junctions with the ovejector the uteri are swollen. The anterior uterus runs straight forward, almost to the cervical glands, where it turns back and proceeds to a point not far posterior to the vulva. Here it turns forward again and having reached the original bend, it terminates in a receptaculum seminis. From this the ovary runs posteriorly to the vulva region, where it may loop several times. The posterior

LEGENDS.

FIG. 1.—Anterior end of male, shewing cervical glands.

FIG. 2.—Vulvar region of female.

FIG. 3.—Tail of female, shewing junction of oviduct and receptaculum seminis.

FIG. 4.—Bursa, ventral view. (Slightly compressed. Accessory piece in lateral view.)

FIG. 5.—Bursa, lateral view.

FIG. 6.—Head, end-on view, shewing lips and papillæ.

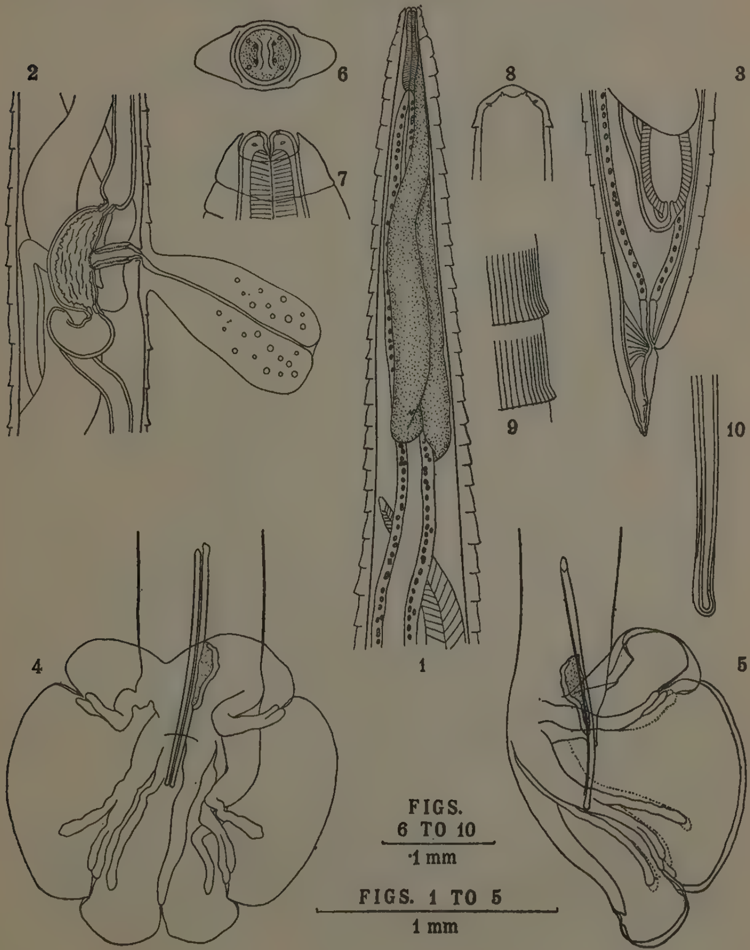
FIG. 7.—Head, lateral view.

FIG. 8.—Head, dorso-ventral view.

FIG. 9.—Cuticular folds and longitudinal ridges in the male.

FIG. 10.—Distal end of spicule, to shew dorsal process and sheath.

uterus loops once after leaving the ovejector and then runs back almost to the anus. Here it doubles forward again as far as the posterior loop of the anterior uterus, where it loops back again and terminates in a receptaculum seminis close to the anus (Fig. 3). The ovary then runs forward to terminate in the same region as the other ovary.



The Larva.

Like the other members of the genus, *C. potos* is viviparous, and by keeping the living females in a watch glass of tap-water, large numbers of larvæ were obtained, which remained alive in this medium for about ten days.

The larvæ are from 0·275 mm. to 0·325 mm. long by about 0·012 mm. in dorso-ventral diameter. Laterally there is a pair of very stout alæ which have their origins about 0·015 mm. from the anterior and posterior extremities of the body, and increase the lateral diameter of the body to about 0·018 mm. The œsophagus is slightly less than half the length of the larva ; in measurements of nine larvæ the value of β ranged from 2·3 mm. to 2·7 mm. It consists of four distinct regions, each with a different diameter. The posterior bulb is the broadest part, and is connected by a long and very narrow part with an anterior region, which is sub-divided unequally by a slight neck into two regions, the anterior being longer than the posterior and slightly thicker.

The intestine is a little shorter than the œsophagus and is connected with the anus by a thin cuticular rectum about 0·02 mm. long.

The tail is from 0·03 mm. to 0·04 mm. long, and half-way along its length it is bent abruptly dorsally at an obtuse angle, in a manner suggestive of the undulating tails of other Protostrongyle larvæ. On some of the larvæ an acicular tip to the tail was observed.

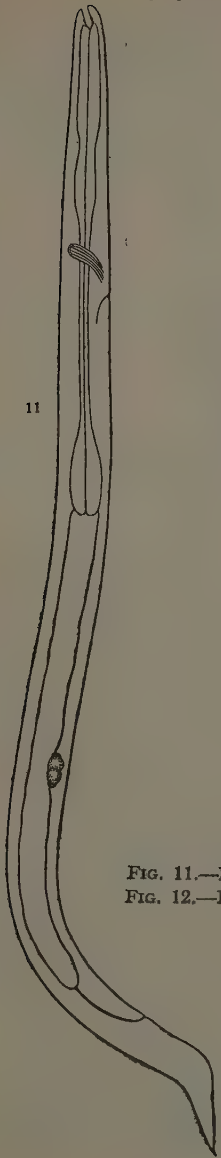
A cluster of cells lying a little anterior to a point half way between the anus and the œsophageal bulb, represents the genital rudiment.

The nerve ring surrounds the anterior end of the narrowest part of the œsophagus, and the excretory pore opens just posterior to the nerve ring.

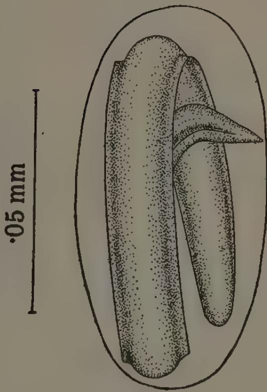
There is a definite buccal capsule. It is cylindrical, about 0·005 mm. long and twice as long as broad. Posteriorly it is encircled by a pair of rugæ. It is tilted dorsally at an obtuse angle to the longitudinal axis of the body, with the result that the mouth opening is antero-dorsal in position.

Discussion.

Reference has already been made to the fact that *C. potos* differs markedly from the other members of the genus. This is very evident in the matter of dimensions, as is shewn in the comparative measurements in the following table :—



11



12

FIG. 11.—Lateral view of larva. (Alæ not drawn.)
FIG. 12.—Egg from uterus, with developed larva.

Male.	*C. striatum.*	*C. vulpis.*	*C. taiga.*	*C. potos.*
Length ...	6·75 mm.	5·0 mm.	7·0 mm.	14·0 mm.
Breadth ...	0·24 mm.	0·3 mm.	0·317 mm.	0·52 mm.
Œsophagus...	0·34 mm.	0·36 mm.	0·3 mm.	0·35 mm.
Spicules ...	0·24 mm.	0·37 mm.	0·294 mm.	0·97 mm.
Ac. piece ...	0·085 mm.	0·55 (?)	0·129 mm.	0·28 mm.
Female.				
Length ...	13·0 mm.	15·0 mm.	13·7 mm.	26·0 mm.
Breadth ...	0·34 mm.	0·46 mm.	0·475 mm.	0·6 mm.
Œsophagus...	0·3 mm.	0·4 mm.	0·55 mm.	0·4 mm.
Tail	0·22 mm.	0·87 (?)	0·16 mm.	0·4 mm.

(The figures in the first three columns are those given by Skrjabin and Petrow (1928). The figures queried are apparently typographical errors and probably should read 0·055 mm. and 0·087 mm.)

The structural distinctiveness of *C. potos* from the other species is evident from the generic diagnosis of Skrjabin and Petrow, in which it is stated, "Mouth rounded . . . bursa consisting of two large lateral lobes and a dorsal lobe." *C. potos* is therefore distinct in possessing lips with papillæ, and a bursa with *four* lobes. Whether these characters should be deemed sufficient for the erection of a new genus for *C. potos*, it is difficult to say, as in all other respects it is obviously closely related to the other species. It is therefore proposed to leave *C. potos* in the genus *Crenosoma*, and to leave the generic diagnosis unaltered pending further data as to the scope of the genus.

The presence of cervical glands in *C. potos* does not constitute a distinctive character. Dujardin (1845), in his description of *C. vulpis* ("*Liorhyncus*" *vulpis*) refers to "deux corps allongés comme les lemnisques des échinorhynques," situated in association with the œsophagus and intestine. There can be little doubt that these bodies are cervical glands.

Similarly, the very striking cuticular swelling at the vulva cannot be put forward as a possible reason for separating *C. potos* from the genus *Crenosoma*, as a similar structure is evidently present, though in smaller form, in *C. striatum* and *C. vulpis*. Molin, in his description of the genus,

includes the following: "Apertura vulvæ in anteriori corporis parte in apice papillæ maximæ, diaphanæ, conicæ," and in the descriptions of both the species such a structure is again referred to. In his figure of the female *C. striatum*, it is plainly to be seen, being in length about half the width of the body at that point.

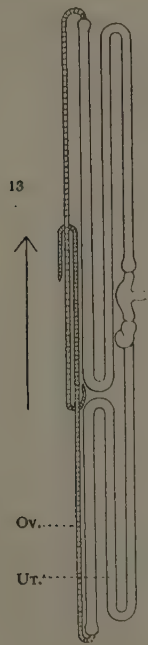


Fig. 13.—Diagram of disposition of female genital organs. (The arrow points anteriorly.)

The writer is indebted to Professor R. T. Leiper for placing the material at his disposal and for much helpful advice.

References.

Dujardin, F., 1845.—" Histoire naturelle des Helminthes ou vers intestinaux." Paris, pp. 112 and 283.

Molin, R., 1861.—" Il sottordine degli acrofalli ordinato scientificamente secondo i risultamenti delle indagini anatomiche ed embriogeniche," *Mem. r. Ist. Veneto di sc. lett. ed arti, Venezia,* Vol. ix., pp. 437-442.

Skrjabin, K. I. and Petrow, A. M., 1928.—" A description of the Genus *Crenosoma* Molin, 1861 (Metastrongylidæ, Nematoda)," *Parasitology*, Vol. xx., p. 329 (*W.L.* 16035).

Zeder, V. G. H., 1800.—Erster Nachtrag zur Naturgeschicte der Eingeweidewürmer mit Zufässen und Anmerkungen herausgegeben, Leipzig, p. 83.

Journal of Helminthology, Vol. VIII, *No.* 4, *December*, 1930, *pp.* 239-248.

On the Infestation of Elm Bark-Beetles (Scolytidæ) by a Nematode, *Parasitylenchus scolyti* n.sp.

By J. N. Oldham, B.Sc., Ph.D.

(Research Assistant, Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.)

Introduction.

Of the insects responsible directly or indirectly for losses in plant industry the Scolytidæ or bark-beetles are well known, particularly to those associated with the practice of forestry, in Europe and America through the effects of their outbreaks. Bark-beetle attacks have resulted in serious damage in many parts of the world although in Britain their depredations have been less severe probably owing to the limited extent of forest lands.

Munro (1926) has written on the general importance of British bark-beetles. He notes that " the majority of our bark-beetles are secondary enemies of trees, their attacks being associated with other adverse influences, and the inter-relations between the bark-beetles and the various influences inimical to the vigorous growth of forest trees is the most important feature of the bark-beetle economy." Apart from the direct damage done by the feeding and boring of these insects indirect losses have been reported principally by an attack by beetles laying open the trees to ravages by various fungi. While the inter-relationship between bark-beetles and fungous attacks has not received adequate attention generally from entomologists and other workers, it is of interest to note that this subject has been brought into prominence through a

report, issued by the Forestry Commissioners in the autumn of this year, on the spread of elm disease in England. This disease, first noticed in Holland and Belgium, is now regarded as being caused by the fungus, *Graphium ulmi*. It has spread over most of western Europe and this year has spread in England to such an extent that its occurrence has been reported from 27 counties. The intensity of the disease varies greatly in different localities and while some places may be very mildly attacked in others the disease sometimes assumes an epidemic character. It has been definitely ascertained that a close relationship exists between the fungus and the Large Elm Bark-beetle, *Scolytus destructor* Ol. This may be observed from the following statement quoted from *The Times* newspaper of November 15th, 1930, page 9, in which a summary of the Commissioners' report is printed.

"The spores are somewhat gelatinous, hence it is improbable that wind plays any great part in dispersal, the more likely agents are birds and insects.

"Of the latter the most important is almost certainly the elm bark-beetle, *Scolytus destructor*, the tunnels of which are nearly always present in the trunks of trees attacked by *Graphium ulmi*. Recent research has shown that the fungus can be isolated from the intestine of the beetle, thus providing evidence of the direct association of the two organisms. In recent months reports have come in showing that there has been a great increase in the numbers of the elm bark-beetle. This is no doubt due partly to the extensive windfalls caused by the gales of the last few winters and partly to the spread of the beetle to trees attacked by the fungus disease. This increase in the beetle population may, and probably does, have a direct bearing on the more rapid spread of the elm disease revealed by the latest survey."

Eradication of the elm bark-beetles or adequate control measures to check their outbreaks would probably contribute towards diminishing or stopping the spread of elm disease apart from counteracting actual damage caused by the insects. Artificial control methods, such as the use of trap-stems, the proper disposal of slash and brushwood and so forth, are used by silviculturalists.

Biological control, which has come into prominence within recent years, has never been favoured against bark-beetles, but it cannot be despised, and there is no doubt that predaceous Coleoptera and parasitic Hymenoptera play an important part in counteracting outbreaks. Of the various natural enemies capable of acting as predators and parasites little has been written, in general, concerning the action of parasitic nematodes upon bark-beetles, while information regarding the sterilising effect

by worms on elm Scolyitids does not appear to exist in scientific literature. From observations made on two species, the Large Elm Bark-beetle, *Scolytus destructor* Ol., and the Small Elm Bark-beetle, *Scolytus multistriatus* Ratz., by the writer in the summer of this year, it was discovered that about 60 per cent. of the insects were parasitised by nematodes. This remarkably high degree of parasitism indicates both the importance of the nematode as a controlling agent and the possibility of it producing considerable reduction in bark-beetle population. The object of this paper is to render an account of the parasite and to indicate its effects upon the hosts.

The material became available through the following circumstances. Several elm trees flanking the drive leading to the Institute's farm at St. Albans were exposed, through the clear-felling of an adjoining strip of mixed woodland, to the severe gales encountered in the winter months of 1929 and 1930. Unfortunately some of the elms were uprooted and overthrown by the force of the wind. They were shortly afterwards trimmed and cross-cut into convenient lengths and allowed to remain lying nearby. The bark having been left on the logs they provided excellent breeding ground for the two elm beetles, the presence of which was noticed in the late spring and early summer of this year. Portions of bark from the logs were removed, placed in a large breeding cage, and as the adult beetles emerged they were collected and examined for parasites. The technique employed in the examination of the nematodes was essentially the same as that outlined by Goodey (1930a), who found Nile blue to act as a useful stain. Although *Scolytus destructor* occurred in much greater numbers than *S. multistriatus*, which normally prefers branches and smaller limbs to the main, thick-barked stems, which only were available in this instance, both species were found to harbour nematodes.

Incidence of Infection.

As mentioned above, *Scolytus destructor* was found breeding to a much greater extent than *Scolytus mulistriatus* and as a result more individuals of the former species were examined than of the latter. In certain cases it was observed that the presence of the nematodes within the body cavity of the beetles had produced the effect of sterilisation of the hosts.

In all some 505 insects were dissected and examined and the results obtained are herewith given.

(*a*) *Scolytus destructor* :—

	Total examined.	Infected.	Infected %	Sterilised.	Sterilised %
Males ...	128	77	60·15	51	39·84
Females ...	257	154	59·92	100	38·52

(*b*) *Scolytus multistriatus* :—

	Total examined.	Infected.	Infected %	Sterilised.	Sterilised %
Males ...	44	26	59·09	17	38·63
Females ...	76	46	60·52	30	39·47

These figures show a preponderance of female over male beetles although the percentage infected and sterilised in both sexes in the two insects are practically equal. Roughly speaking 60 per cent. of the beetles harboured nematodes and 40 per cent. of them exhibited sufficient evidence of reduction in the size of the reproductive organs to classify them as sterile.

These figures show that the parasite is exercising an appreciable controlling effect upon the bark-beetles and is of importance therefore not only in holding them in check but probably in reducing their numbers in successive generations.

Effect on the Host.

The reproductive organs of both male and female individuals of *Scolytus destructor* and *S. multistriatus* were seen to be greatly reduced in size and the impression gathered was that, in all probability, they would be non-functional. The parasites were all found within the body cavity in the abdominal region and in no case were any encountered within the thorax or head. Likewise, the examination of the dissected-out alimentary canal showed that no worms were present within it. It was rather difficult to estimate whether damage to the fat tissue had occurred and in the majority of specimens this exhibited a normal appearance. Dr. Fisher, who studied the biology of *Scolytus destructor* Ol. during 1921 to 1924 in the London district, has communicated (*in litt.*) his experiences to the writer to the following effect. Nematodes

were of frequent occurrence in the insects and when dissecting out the reproductive organs large numbers of worms were obtained from the body cavity and, in female beetles, the *bursa copulatrix* was frequently distended with nematodes. Dr. Fisher has studied the effect of the presence of the parasite upon the duration of the feeding period of the immature beetles and intends to publish his observations along with biological studies on *Scolytus destructor* in the early part of 1931.

Description of the Parasite.

(*a*) *Larva within the Beetle.*

The larvæ recovered from the abdominal cavity of the insect measure from 0·56 mm. to 0·66 mm. in length by 0·022 mm. to 0·027 mm. in

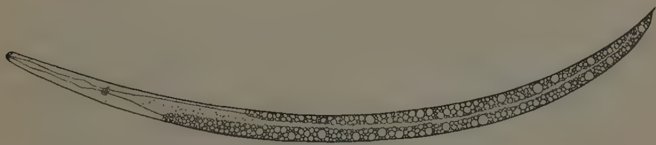


Fig. 1.—Larva of *Parasitylenchus scolyti* n. sp., from body cavity of *Scolytus destructor* Ol.

greatest width. The most striking feature about them is that they are almost completely filled with large numbers of fat globules, which showed up remarkably clearly when stained with Nile blue, thus almost completely obscuring the internal anatomy of the nematode. The body tapers slightly anteriorly and posteriorly. The head is simple lacking any constriction, evident lips or papillæ, while the tail tapers off, ventrally, into a very fine point. The mouth is terminal leading into a narrow œsophagus. No stylet or spear appears to be present. The œsophageal region, not quite uniform in diameter, exhibits a weakly swollen appearance about 0·06 mm. from the anterior end. Thereafter it narrows and is followed by a gradually widening intestinal region whose limits become lost in the mass of fat globules. Posteriorly, the intestine is connected by a short rectum to the anus which lies 0·026 mm. from the tip of the tail. Whether such larvæ as just described arose from eggs from the

parasitic female, dealt with below, or whether they would develop further and become adults parastitic in the beetle or escape to the exterior to form a free-living generation has not as yet been ascertained. It is intended, however, to pursue the study of the life history of this parasite in detail during the forthcoming season and to establish more fully the various findings which have hitherto been made.

(*b*) *Parasitic Female.*

During the examination of a large number of beetles only one adult female was recovered. On this specimen the following account is based. In general appearance the worm is sausage-shaped measuring 0·82 mm. in length and 0·08 mm. in width and lies free in the abdominal cavity of its host. Owing to somewhat indifferent preservation contraction of head and tail ends occurred, producing a rather annulated appearance in these regions. There appears to be indications of a stylet the exact character of which was difficult to observe in the fixed specimen. A somewhat granular region, representing the alimentary canal, can be distinguished. This extends backwards to become overlaid by the forwardly directed ovary in which separate cells can be distinguished. The ovary gives place to the oviduct which attains a length of about one-third of the total body length and increases slightly in width as it proceeds rearwards. At about the middle of the body a distinct swelling occurs which, owing to its granular appearance, leads one to consider this as being the receptaculum seminis. From this point onwards the uterus, containing a number of ova, extends and terminates at the vulva,which is situated at a short distance from the tip of the tail. No clear indication of the presence or position of the anus could be discerned. The cuticle appears to have stretched in order to accommodate the developing internal organs, and dispersed throughout the body-wall can be seen isolated cells containing enlarged nuclei.

Other Records of Nematodes in Scolytids.

Zwaluwenburg (1928) lists ten species of Scolytids as having been recorded to harbour nematodes. The majority of the parasites have been discovered by Fuchs (1914a, 1914b, 1915, 1929) who has in nearly all cases used a trinomial system of nomenclature. Only one other record so far is known to the writer dealing with parasitism of bark-beetles in which Gavalov (1926) found the pupæ of *Scolytus scolytus* F.,

(= *destructor* Ol.) in the Crimea to harbour nematodes. Fisher's unpublished studies have already been noticed in a preceding section.

Allusions to parasitic castration in the Scolytidæ are made by Jazentkovsky (1924), who records the presence of nematodes in larvæ (2 to 6 per cent.), pupæ (25 per cent.) and adults (25 per cent.) of the Pine-shoot Beetle, *Myelophilus piniperda* L., and the Lesser Pine-shoot Beetle, *M. minor* Hart. injuring the reproductive apparatus of both

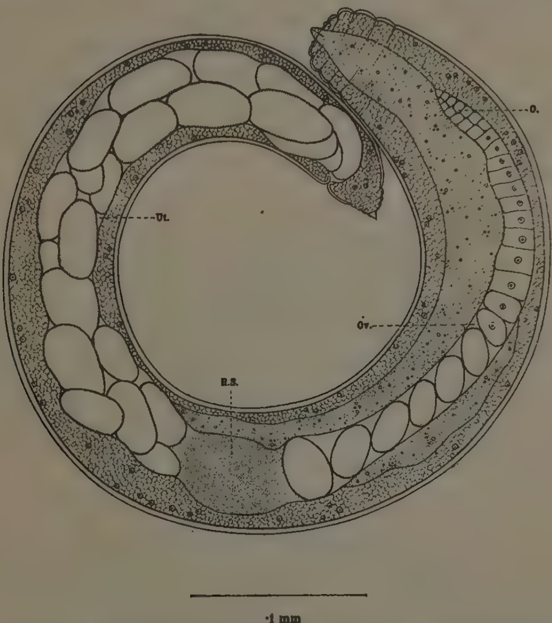


Fig. 2.—Parasitic female of *Parasitylenchus scolyti* n. sp. from the body cavity of *Scolytus destructor* Ol.

(R.S.=Receptaculum seminis; O=Ovary; Ov=Oviduct; Ut.=Uterus).

sexes. Fuchs (1914b) also reports the presence of *Tylenchus contortus typographi* and *T. dispar typographi*, both of which live in the body cavity of their host, in *Ips typographus* L. Their effect upon the host is to lower its general activity and in particular its egg-laying capacity;

he mentions that healthy beetles lay on an average 60 eggs in a batch, while parasitised individuals lay only about half this number.

Systematic Position.

The majority of the parasitic nematodes recorded from bark-beetles have been found by Fuchs, who has placed them in the genus *Tylenchus*. Micoletzky (1921), however, erected the genus *Parasitylenchus* to receive those species which Fuchs had described up to 1921. This new genus appears to have been accepted and used by Wülker (1929) in creating a new species, *Parasitylenchus cossoni* from the Curculionid, *Cossonus parallelipipedus* Hrbst. At the same time Wülker criticises Fuchs from the systematic standpoint upholding the retention of the genus *Parasitylenchus*.

In several respects the parasite found by the writer is similar to those described both by Fuchs and Wülker and, lacking an adequate number of representative forms in which a critical examination of morphological characters can be made, it is considered advisable for the present, at least, to assign it to the genus *Parasitylenchus*. As it does not, however, wholly conform to the description of any other nematode described from Scolytidæ the writer has felt justified in establishing a new species and having discovered it in two bark-beetles belonging to the same genus proposes for it the name of *Parasitylenchus scolyti* n. sp.

An Associated Free-Living Nematode.

During the examination of the beetles numbers of free-living nematodes were found exteriorly on the bodies of pupæ and adults. They tended to congregate in clusters and were most numerous under the elytra of both stages of the insect, but were also seen on the abdomen around and between the coxal joints. Examination of these forms disclosed them to be individuals of *Cylindrogaster ulmi*, described by Goodey (1930b), who obtained his specimens, by the Baermann extraction method, from some debris taken from beneath the bark of an elm tree in which the bark-beetles were breeding and from which the writer obtained his insect material. This observation is of interest in that it directs attention

to the possibility of an external mechanical association existing between worm and beetle,the former using the latter as a carrier for transportation to new localities or food-supplies.

REFERENCES.

FISHER, R. C., 1928.—" The Relation of the Elm Bark-Beetles to their Host Trees," *Forestry*, Vol. II, No. 1, pp. 53-61.

FUCHS, G., 1914A.—" *Tylenchus dispar curvidentis* m. und *Tylenchus dispar cryphali* m." *Zool. Anz.*, Vol. XLV, No. 5, pp. 195-207. (*W.L.* 23833).

— 1914B.—" Ueber Parasiten und andere biologisch an die Borkenkäfer gebundene Nematoden," *Verh. Ges. deuts. Naturf. Ärzt.*, Vol. LXXXV, No. 2, Part 1, pp. 688-692. (*W.L.* 22277.)

— 1915.—" Die Naturgeschichte der Nematoden und einiger anderer Parasiten (1) des *Ips typographus* L. (2) des *Hylobius abietis* L.," *Zool. Jahrb.*, Abt. Syst., Vol. XXXVIII, Nos. 3 and 4, pp. 109-222. (*W.L.* 23831).

— 1929.—" Die Parasiten einiger Rüssel- und Borkenkäfer," *Z. Parasitenk.*, Vol. II, No. 2, pp. 248-285.

GAVALOV, I. I., 1926.—" Some injurious Beetles from the Crimea (Col.)," (in Russian). *Acta Soc. ent. Stauropol*, ii., pp. 39-42.

GOODEY, T., 1930A.—" On a Remarkable New Nematode, *Tylenchinema oscinellæ* gen. et. sp. n., parasitic in the Frit-fly, *Oscinella frit* L., attacking Oats," *Philos. Trans.*, Ser. B, Vol. 218, pp. 315-343. (*W.L.* 16192).

— 1930B.—" A New Species of the Nematode genus *Cylindrogaster*," *J. Helm.*, Vol. VIII, No. 2, pp. 89-92.

JAZENTKOVSKY, A., 1924.—"Kastration der Waldgärtner von Würmern (Nematoden) und ihr Einfluss auf die Lebensfähigkeit der Borkenkäfer, Ipidæ, vorlänfiger bericht," *Mem. Inst. Agronom. d'Etat de la Belrussie*, liv. 3, pp. 278-296 (in Russian with German summary). Abstract in *Zbl. Bakt.*, IIte Abt., Vol. LXIX, No. 1-7, pp. 157-158, Nov., 1926. (*W.L.* 23684.)

MICOLETZKY, H., 1921.—" Die freilebenden Erd-Nematoden," *Arch. Naturgesch.*, 87 Jahrg., Abt. A, Nos. 8 and 9, p. 545. (*W.L.* 1782).

Munro, J. W., 1926.—" British Bark-Beetles," ***Forestry Commission Bulletin No. 8***, pp. 77. London.

Wülker, G., 1929.—" Bemerkungen zur Arbeit von G. Fuchs : " Die Parasiten einiger Russel- und Borkenkäfer,' " ***Z. Parasitenk***, Vol. ii, No. 2, pp. 286-290.

Zwaluwenburg, R. H. van, 1928.—" The Interrelationships of Insects and Roundworms," *Bull. H. S. P. A. Expt. Sta.*, Entom. Ser. No. 20, pp. 36-37.

Index to Vol. VIII.

Index of Authors.

New Names in Volume VIII.

New Species.